TMA SUBMISSION

ETS AND LUNG CANCER

SCOTH REVIEW 1994

(VOLUME 3)

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ETS AND LUNG CANCER - SCOTH REVIEW

(VOLUME 3)

CONCLUSION

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TMA SUBMISSION

SCOTH REVIEW - VOLUME 3

CONCLUSION

The ETS and lung cancer epidemiology has been the main basis for the claim that ETS can cause lung cancer in non-smokers. To date, there have been some 37 epidemiological studies on ETS and lung cancer which have been sufficiently well reported to justify their consideration. The vast majority of these studies did not report overall statistically significant results and thus chance cannot be ruled out as an explanation for their findings. Those which have reported marginally significant results have generally failed adequately to account for alternatives to the causal hypothesis. When all of these studies are considered, and even when meta-analysis is applied, the epidemiological data does not support an inference of causality or even of genuinely elevated risk.

Other published work has addressed the plausibility of the claim based on biological or chemical information. There is no agreement on a definition of ETS or how it should be measured. Since measurements that have been made show significant chemical and physical differences between MS, SS and ETS, the claim that ETS poses health risks to non-smokers cannot be supported by extrapolation from the reported risks of MS. Attempts have been made to quantify exposure to ETS, but the only method that has been used widely in the epidemiological studies involves questionnaires, which is demonstrably seriously flawed. Alternative assessments of exposure have included ambient measurements of various chemicals, but these techniques have also been reported to be problematic. Recent studies using personal monitoring of tobacco-specific components have reported a much lower level of exposure than has often been postulated, and highlight the difficulty of subjective assessment.

Biological markers have also been used to assess exposure to ETS, but the problems of such assessment are substantial. At best, the use of such markers has highlighted the surprising degree of misclassification of smokers as non-smokers, which has important implications for the interpretation of the ETS and lung cancer epidemiology.

Overall, the scientific evidence does not support the claim that ETS exposure is a cause of lung cancer in non-smokers.

TMA

August 1994

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SECTION 1

ETS AND LUNG CANCER

INTRODUCTION

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SCOTH REVIEW - VOLUME 3

INTRODUCTION

The material being submitted by the TMA for this review follows the request of the SCOTH Secretariat - namely concentrating on peer-reviewed science, published since the Fourth Report of the ISCSH (1988) or not taken into account in their report.

This volume follows on from the previous two volumes, which between them cover issues related to epidemiological studies on ETS and lung cancer. Such studies have been the main basis for the claim that ETS can cause lung cancer - however our contention in the previous volumes was that the epidemiological studies do not support this claim, whether considered individually or in combination through meta-analysis.

Other published work has addressed the plausibility of the claim, based on biological or chemical information. This volume provides materials on these aspects. Section 2 provides studies on the chemistry and physics of mainstream smoke (MS), sidestream smoke (SS) and ETS, showing how different they are both quantitatively and qualitatively.

Virtually all of the epidemiological studies used questionnaires to assess exposure, usually involving the recollection of a relative. Section 3 contains studies showing the inherent problems in the accuracy of such reported exposures. Personal monitoring studies are included, and these highlight some of the errors in questionnaire-based assessment. The degree of misclassification of smokers reported to be non-smokers, documented in Volume 2, has been shown in some of the studies cited here to be rather high, and this influences the interpretation of epidemiological studies.

The uptake of chemicals present in ETS is not well understood, and the US EPA commented that it was too early for studies on this subject to be useful. One result of cotinine measurements in "non-smokers" has, however, been to identify an unexpectedly high degree of smoker/non-smoker misclassification. Section 4 provides some such studies, including a wide-ranging unpublished review of relevant literature which was commissioned by the TMA.

We are not aware of any laboratory animal ETS inhalation studies. ETS is too unstable a mixture, and its real life concentration too low, for it to be suitable for such studies. Despite the differences between ETS and (SS), acute and chronic exposure studies have been carried out using aged and diluted SS as a surrogate for ETS in animal inhalation tests. The studies, included in Section 5, reported no irreversible effects, and only limited changes of any sort, and these are unrelated to events in the multistage process of carcinogenesis.

Section 6 comprises a review of many of the aspects covered in these sections.

The overall evidence, as presented in the three volumes, is summarised briefly in the conclusion immediately preceding this introduction.

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SECTION 2

ETS AND LUNG CANCER

COMPOSITION

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SCOTH REVIEW - VOLUME 3

SECTION 2

COMPOSITION

Although there is no stable chemical definition of ETS, it is clear that it differs substantially from MS and SS. ETS can be defined as a continually changing, ageing, and heavily diluted mixture of SS and the remnants of MS exhaled by smokers.

The papers in this section discuss the composition of ETS, and highlight the chemical and physical differences between ETS, MS and SS[1].

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[1] Further discussion of this issue can be found in Guerin et al, *The Chemistry of Environmental Tobacco Smoke* which is submitted under separate cover.

SECTION 2

COMPOSITION

REFERENCES

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Baker & Proctor (1990). The Origins and Properties of Environmental Tobacco Smoke. Environment International

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*Guerin et al (1992). In, The Chemistry of Environmental Tobacco Smoke, edit Eisenberg, Chapters 3 & 4. Submitted under separate cover.

* Not peer reviewed

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The Aging of Sidestream Tobacco Smoke Components in Ambient Environments

R. R. Rawbone, W. Burns, and G. Haslett

Summary

A large number of sidestream smoke components have been measured over a 50-min time period in a well-defined experimental room. The results show a variable rate of decay following smoking which would suggest that extrapolation from a single measured "marker" to other potential smoke components should be performed with caution.

Introduction

Environmental tobacco smoke is a dynamic aerosol and its characteristics, both physical and chemical, depend on a number of factors; these include the elapsed time since its formation, whether the smoke plume is allowed to fully form before dispersion and the more general dilution within the ambient environment [1]. In terms of a single point sampling site the resultant measurement value will therefore not only depend upon the characteristics of the environment and the number and manner of cigarettes being smoked but also upon both temporal and spatial factors of the sampling position relative to the smoking.

This dynamic nature of the aerosol results not only in a loss of volatile components, including nicotine, from the particles to the vapour phase, but also in a complex and variable behaviour of the individual chemical components which manifests in their exhibiting different decay characteristics. This is of importance in the interpretation of ambient air studies which are generally limited to the measurement of one or two environmental tobacco smoke markers.

The objective of this paper is to demonstrate this variability in decay patterns for a series of chemical measurements over a 50-min period following smoke generation in a well defined experimental room.

Materials and Methods

Smoke was generated using a modified smoking head from a Battelle rotary smoking machine [2] in a specially constructed room with a volume of about 48,000 litres. The internal walls, ceiling and floor were coated with a sealant paint and there was a single door with no windows, other than a sealed observation port; all other access, including that for electricity supply and air sample collection, was through sealed ducting. During the current studies there was no active ventilation in the room and furniture was kept to a minimum. Temperature and humidity were monitored continuously.

H. Kasuga (Ed.) Indoor Air Quality

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At the start of each experiment 16 cigarettes, with a standard mainstream delivery of 17 mg tar (PMWNF), were smoked on the rotary smoker to the reference conditions of one 35 ml puff of 2 s duration every minute. The mainstream smoke was ducted away and the sidestream smoke, after formation of the plume, mixed into the room by a series of fans. In order to maintain a constant carbon monoxide level in the room throughout a 50-min-study period, as a standard condition, single cigarettes were smoked subsequent to the initial 16 cigarettes being extinguished. The time at which the initial cigarettes were extinguished was also taken as time zero for the commencement of chemical measurement.

Ambient chemistry in the room was measured using the following techniques which have also been employed, for comparative purposes, in a benchtop collection device [3] for the measurement of freshly generated sidestream smoke:

Carbon monoxide was measured continuously using a non-dispersive infra-red analyser (Analytical Development Co., Model RFA/1).

Nicotine, which is distributed between the particulate and vapour phases, was measured as total nicotine by collection into a Tenax trap over 5-min-sampling periods, with subsequent thermal desorption and gas chromatographic analysis (Perkin Elmer, ATD50).

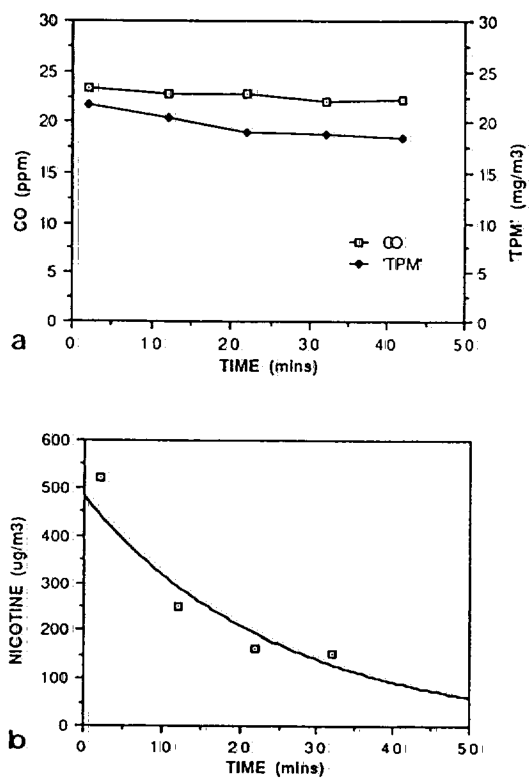

Fig. 1. a Changes in ambient concentrations of carbon monoxide and "Mini-ram" particulates. b Changes in ambient concentration of nicotine

Particulates were measured using the MINIRAM (Miniature Real-time Aerosol Monitor, GCA Corporation, Model PDM-3), a light scattering device which samples over 10-s-time periods. This instrument gives a quantitatively high result because of its sensitivity to particle size distribution and its dependence upon a relevant calibration [4].

Ammonia was measured continuously using a selective ion electrode.

A "whole smoke" gas chromatographic profile was obtained by actively drawing the ambient atmosphere through standard Perkin Elmer ATD50 tubes packed with Tenax TA, 60–80 mesh for a 15-min-period at a flow rate of 300 ml/min. The Tenax was then thermally desorbed in two stages onto a 50 m mixed Ucon phase capillary column. Values for 33 distinct peaks were calculated as the peak area relative to that of the Internal Standard (Dimethyl Furan), these included Acetone, Acrolein, Acetonitrile, Pyridine and 3-vinyl pyridine.

A "phenolic profile" was obtained by drawing the atmosphere through a small Cambridge filter pad for 10 min at a flow rate of 20 l/min. The pad was then silylated using BSTFA and Digol was added as an Internal Standard. This was then heated for 1 h at 80°C and run on a 25 m SE54 capillary column. Values for 26 peaks, including Catechol, Glycerol, and Hydroquinone were calculated with reference to the Internal Standard.

Results

Figures 1a and 1b show the results for nicotine, carbon monoxide and Miniram particulates. The carbon monoxide levels remain constant at the relatively high level of 22 ppm throughout the 50-min-study period, this being consequent upon the defined

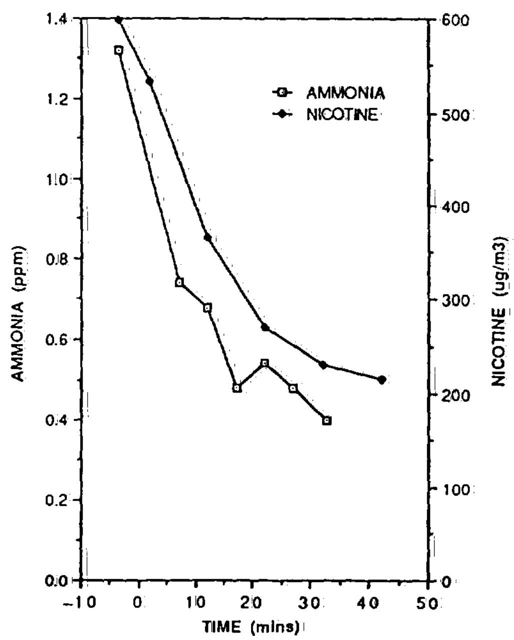

Fig. 2. Changes in ambient concentration of nicotine and ammonia

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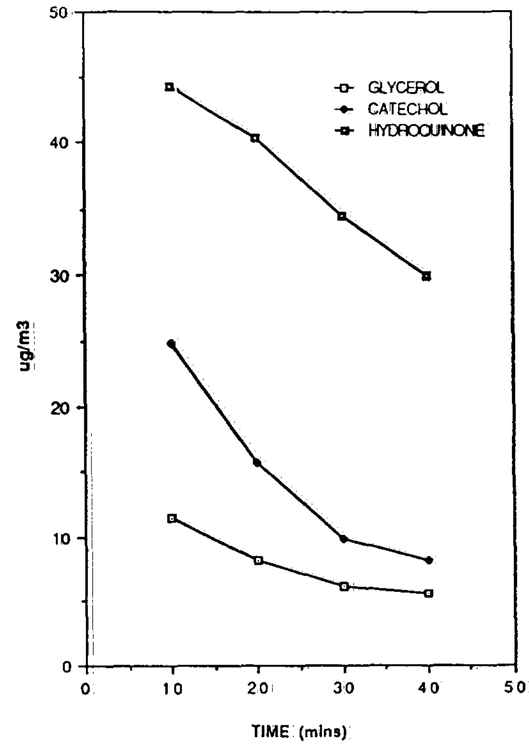

Fig. 3. Changes in ambient concentration of Catechol, Glycerol and Hydroquinone

smoking regimen. The particulate levels can be seen to fall by about 15% and this is most likely accounted for by the loss of volatile materials to the ambient atmosphere. In contrast to this relatively small decline in particulate levels however is the rapid fall in airborne nicotine levels which decay to less than 20% of their initial value.

Figure 2 shows that the levels of ammonia exhibit a similar rapid decay to that seen for nicotine.

Examples from the analysis of the "phenolic profile" are given in Fig. 3 which illustrates the decay of Catechol together with Glycerol and Hydroquinone. These results draw attention to the fact that whilst the majority of components appear to show an exponential decay pattern this is not invariable and as an example Hydroquinone appears to decay over this time period in a linear fashion.

Because of the longer periods over which the "whole smoke" profile samples are obtained it is not possible to display the changes graphically. Comparing the time periods 0–15 min with 30–45 min gives some idea of the variability in rates of decay. These are illustrated in Table 1 where the percentage change of individual peak areas between the two periods can be seen to range from 0% to 40%.

Of the 50 plus components of sidestream smoke examined in these studies in no case was any component found to increase over the 50-min-time period.

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Table 1. Levels of major components in the "whole smoke" profile of an ambient air sample and their % change over a 45-min-time period

Peak No.	Identification	15–30-min-value	45–60-min-value	% change
1		1.51	1.64	− 5
2		0.62	0.61	−13
3		4.40	3.94	−22
4		0.65	0.74	0
5		0.52	0.46	−22
6		0.36	0.30	−27
7	Acetone	0.77	0.87	0
8	Acrolein	0.34	0.38	− 3
9		1.28	1.22	−16
10	Pyridine	1.05	1.10	− 8
11	Acetonitrile	0.67	0.68	−11
12		0.68	0.58	−25
13	Benzene	2.66	2.36	−22
14		0.52	0.53	−10
15	*Int. Standard*	1.00 (33.5)	1.00 (29.4)	
16	Toluene	4.51	5.20	0
17		0.79		
18		2.31	1.93	−27
19		2.29	1.73	−34
20	3-Vinyl pyridine	0.70	0.76	− 5
21	Phenol	1.99	2.28	0
22		2.25	1.75	−32
23		0.89	0.75	−26
24		1.20	1.00	−27
25		1.31	1.23	−17
26		3.53	3.00	−25
27		1.92	1.90	−13
28		1.45	1.40	−15
29		1.83	1.56	−25
30		2.01	1.68	−27
31		1.64	1.25	−33
32		1.02	0.74	−36
33		0.65	0.44	−40

Values presented were calculated by the (peak area of component)/(peak area of Internal Standard). Values in brackets were the actual peak areas. The % change between the results allows for the differences in value for the Internal Standard

Discussion

The results presented in this paper clearly demonstrate the variability in the decay pattern for individual components of environmental tobacco smoke. Although the measurements were made in an experimental situation at a relatively high ambient smoke level this variability would certainly be encountered in the real-life situation.

It is thus clear that to make extrapolations from the measurement of a single marker to the behavior of other smoke components involves an assumption which is likely to be

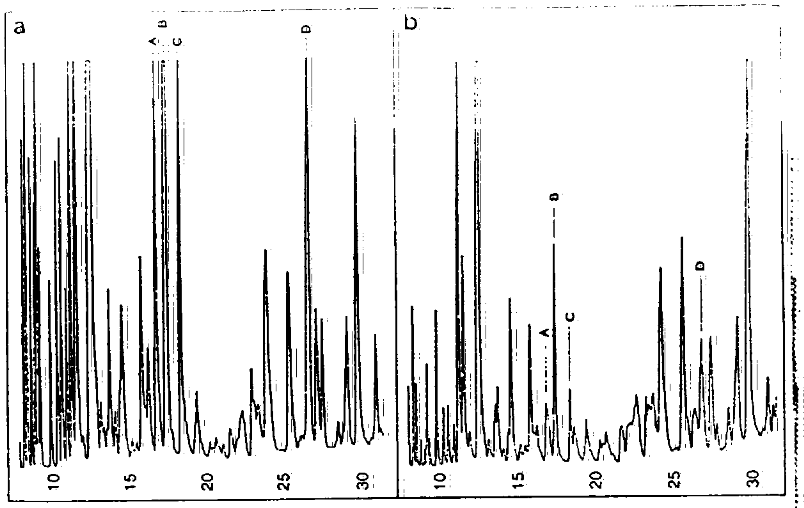

Fig. 4a, b. "Whole smoke" chromatographic profiles of (a) freshly generated sidestream smoke in a benchtop apparatus and (b) environmental tobacco smoke following the dispersion of sidestream smoke from 16 cigarettes

invalid. One further point can be noted from a comparison between fresh sidestream smoke measured in a benchtop apparatus and sidestream generated in the experimental room. This is illustrated in Fig. 4 which presents the whole smoke profiles obtained in a Keith apparatus with that taken in the experimental room immediately following the smoking of the 16 cigarettes. The four components labelled A, B, C and D, which have been provisionally identified as Furan, Acetone, Acrolein and Acetonitrile, are among those which can be seen to have greatly reduced levels in the room relative to those in the benchtop collection device. Although these components appear in fresh smoke their apparent decay is so rapid that they may not be seen to any significant extent in room air.

Conclusions

1) Environmental tobacco smoke is a dynamic aerosol which exhibits both temporal and spatial variation.
2) Each of the components of smoke measured has its own decay rate and pattern. Relative to carbon monoxide and particulates, nicotine and ammonia have rapid decay rates.
   Other components, which probably include Acrolein, decay at an even faster rate and high airborne levels are probably never achieved.
3) Extrapolations from benchtop sidestream measurement to room air based on simple dilution calculations is unlikely to provide valid information.
4) Extrapolations from a measured "marker" in ambient air studies to other potential smoke components should be performed with caution.

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THE ORIGINS AND PROPERTIES OF ENVIRONMENTAL TOBACCO SMOKE

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EI 8906-186M (Received 16 June 1989; accepted 1 December 1989)

Environmental tobacco smoke (ETS) is formed from cigarettes as sidestream and exhaled mainstream smoke diffuse into ambient air. Detailed studies are reviewed which describe how sidestream smoke is formed, its acceleration away from the cigarette and its chemical properties. As the smoke streams diffuse into the atmosphere they become greatly diluted and physical and chemical changes occur. A quarter of the material in sidestream particles evaporates, so that ETS nicotine is virtually entirely in the vapour phase, and the particles shrink. As cigarettes are smoked, the levels of ETS components rise and then fall exponentially due to air exchange and deposition of smoke particles onto surfaces. The decay of ETS also depends on the particular component, with nicotine decaying faster than other substances. In real-world environments, ETS is found along with chemicals and particles from many sources. Studies are reviewed which quantify the contribution of ETS to various indoor air environments. These include determination of the ETS proportion of total respirable particles, measurement of nicotine as a specific ETS marker, and comparisons of chemicals present in matched smoking and nonsmoking environments. The ETS contribution of volatile organic compounds in air is much less than that from other sources. The review emphasises the need for tobacco specific analytes to be used as ETS markers and/or to apportion ETS particulate matter from total particulate matter in the atmosphere.

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INTRODUCTION

Environmental Tobacco Smoke, ETS, has received considerable attention in recent years. ETS is the complex mixture of chemicals found in air as a specific result of tobacco smoking (Nystrom and Green 1986). Some reports have claimed that exposure to ETS can be harmful to the health of nonsmokers (USSG 1986; NRC 1986; ISCSH 1988). This issue has been discussed by scientists and epidemiologists for over a decade and although knowledge has increased over this period, it is still the subject of scientific controversy (Mantel 1987; Uberla 1987).

In order to assess ETS properly it is necessary to understand some of its chemical and physical properties, and to ascertain the concentrations of ETS present in typical environments (Repace 1987b; Proctor et al. 1989a).

The aims of this paper are to describe the origins of ETS through an understanding of the combustion processes occurring within a cigarette, how it builds up and then decays in indoor air, and some of its properties. This paper considers ETS in relation to cigarettes, rather than that originating from cigars and pipes.

THE ORIGINS OF ETS

ETS results from a combination of sidestream smoke (that which is released from the lit end of the cigarette), and exhaled mainstream smoke (that exhaled by the smoker after drawing on the cigarette), both greatly diluted by the ambient air.

Fresh sidestream smoke

Sidestream smoke is defined as all the smoke generated by a cigarette that is not mainstream. Side-

stream smoke is made up of the sidestream plume which is emitted from the burning zone during both the puff and smoulder periods in an upwards direction (because of buoyancy), the smoulder stream which escapes from the mouth end of the cigarette during smoulder, and gases which diffuse out of the tobacco rod by diffusion during both the puff and smoulder periods (Lipp 1965; Hoegg 1972; Baker 1982). It has been estimated that the sidestream plume contributes about 95% to the total sidestream smoke (Hoegg 1972).

Physical and chemical aspects of the sidestream plume in the vicinity of the burning zone of a smouldering cigarette are illustrated in Fig. 1. (In both Figs. 1 and 2, % v/v is % volume/volume. It should be noted that 1 % v/v = 10 mL/L). The data in this figure were measured in a variety of experiments previously described (Baker 1982; Robinson 1987). The position of the sidestream smoke plume was obtained by carefully photographing the smouldering cigarette under controlled air flow conditions around the cigarette. The position of the gas phase plume was obtained in a series of experiments in which small sampling probes were placed around the cigarette and connected directly to a mass spectrometer. The temperature distribution was measured using small thermocouples placed around the burning zone. The velocity distribution was measured using a laser Doppler velocimeter technique.

The gas phase temperature distribution outside the cigarette is very similar to that obtained earlier by Neurath et al. (1966). The positions of the smoke and gas phase plumes have also been confirmed using a Schlieren optical method described by McRae and Jenkins (1987).

In the smoulder period between the puffs, a natural convection flow of air around the burning zone in an upwards direction (because of buoyancy) sustains burning but at a much lower intensity than during the puff. Little change occurs to the external temperature and oxygen distributions when the puff is taken (Baker 1982), indicating that the natural convection stream around the burning zone and into the sidestream plume is only slightly affected by the influx of air during the puff. The combustion processes occurring on the surface of the burning zone in the convection stream proceed independently of those inside.

The main products in the gas plume are carbon monoxide, carbon dioxide, hydrogen and water — the concentration distribution of carbon monoxide outside the burning zone during smoulder is shown in Fig. 1 and the forms of the profiles of the other gases and oxygen depletion are similar. The carbon monoxide plume originates some 3 to 4 mm in front of the paper burn line, as do the external temperature contours. The carbon monoxide concentration immediately above the burning zone is higher than that just inside, and a similar situation exists for carbon dioxide. Thus, sidestream carbon monoxide and carbon dioxide are not formed only by the combustion products diffusing out of the burning zone — some must be formed on the external surface from reactions with the oxygen convected around the coal.

Fig. 2 illustrates the variations during the smoking cycle of the gas concentrations of oxygen, carbon dioxide, carbon monoxide and hydrogen at a specific point in the sidestream gas plume. This point is situated 1 mm above the surface of the burning zone, and 3 mm in front of the paper burn line. The plume hydrogen and carbon monoxide concentrations at this point increase during the puff while the carbon dioxide concentration falls. At the end of the puff, the level of all three products increases for about one second. This variation is very similar to that found inside the centre of the burning zone (Baker 1981), and is due to the outward diffusion of those products formed inside the burning zone. The small rise in carbon monoxide and fall in carbon dioxide during the puff are due, at least partly, to the carbonaceous reduction of carbon dioxide to the monoxide, which occurs as the temperature in the interior of the burning zone increases as the puff progresses. When the puff ends, the product formation-transmission balance inside the burning zone is interrupted, resulting in a local build-up of gases in their formation regions. These diffuse into the sidestream to deplete the local build-up.

In contrast to the gas phase plume, the sidestream smoke plume originates 0-4 mm behind the paper burn line, becoming visible at temperatures below about 150°C (Fig. 1). This is the approximate position of the tobacco pyrolysis and distillation region inside the cigarette (Baker 1981). Inside the cigarette in this region, a concentrated organic vapour is formed. During the smoulder period much of it will diffuse radially out of the cigarette through the partially degraded cigarette paper, although some will also diffuse axially towards the mouth end of the cigarette to form the smoulder stream. As the vapour diffuses through the paper to the outside, it is subjected to a sudden temperature decrease and dilution. These conditions favour the formation of relatively small aerosol particles compared to mainstream particles.

Since the sidestream plume is the major contributor to ETS, the rate at which sidestream smoke is transported away from the cigarette into the atmo-

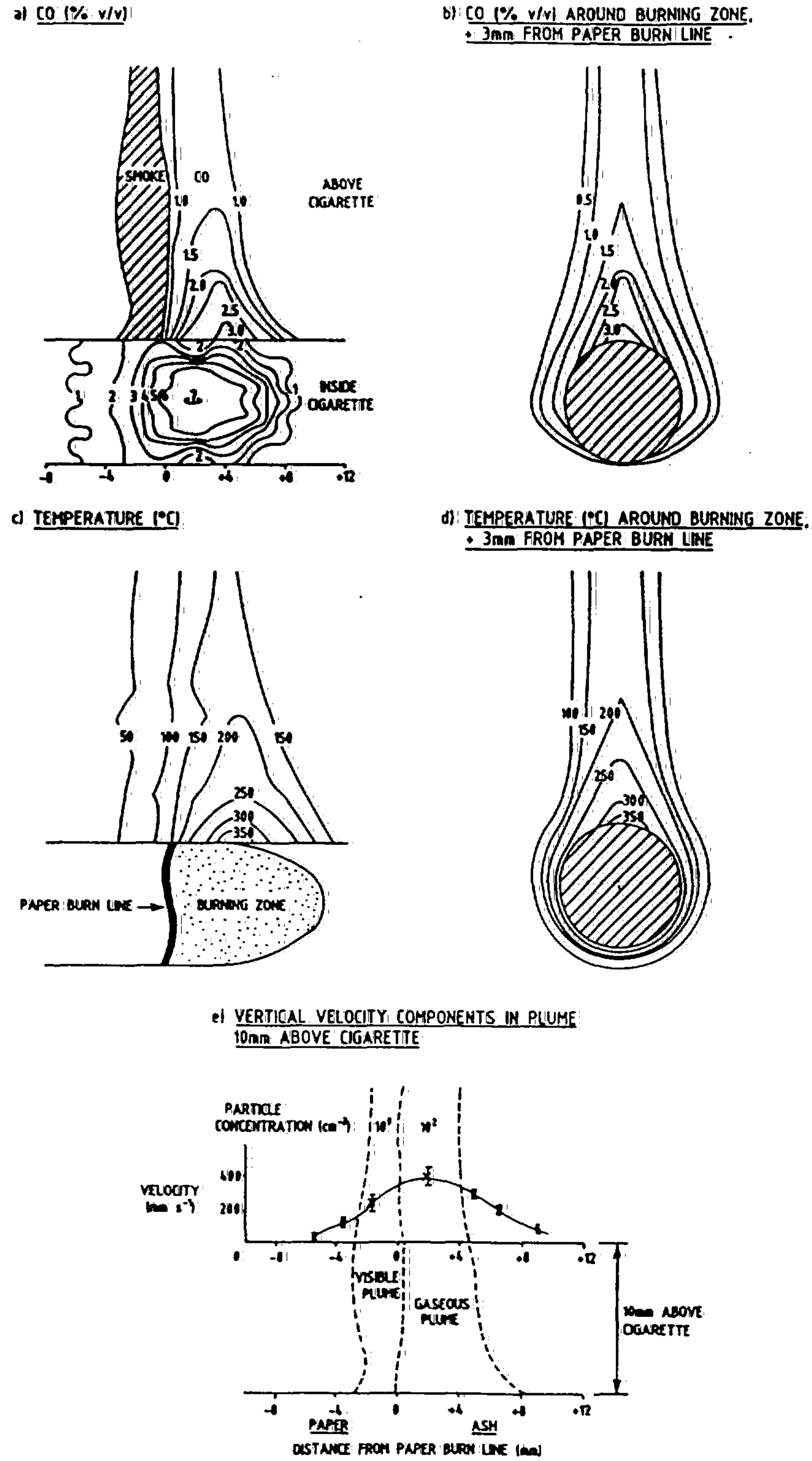

Fig. 1. Concentrations, temperatures and velocities in sidestream plume during smoulder (Baker 1982; Robinson 1987).

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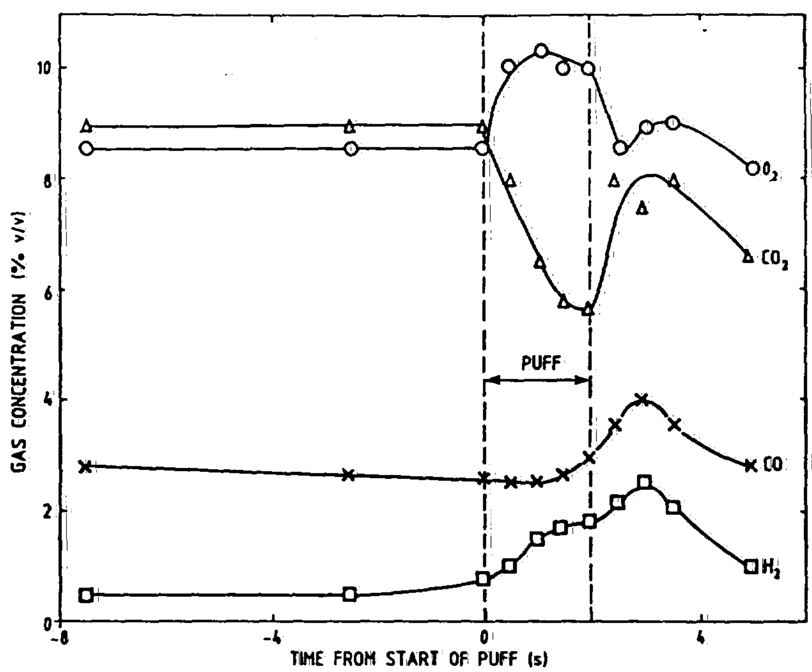

Fig. 2. Variation with time of sidestream gas concentration 1 mm vertically above cigarette and +3 mm from paper burn line.

sphere ultimately determines the rate of build-up of ETS components. A detailed determination of the velocity distribution of the sidestream plume has been made using a laser Doppler velocimeter described by Robinson (1987). In this technique, two laser beams are focused on a given point in the plume and produce an interference pattern at their point of intersection. Aerosol particles carried in the plume scatter light from the interference pattern and characteristics of this light can be used to calculate the velocity of the particles in the plume. The results indicate that at a given distance above the cigarette there is a distribution of velocity. That obtained at 10 mm above the cigarette is shown in Fig. 1(e) together with an estimate of the particle concentrations in the gas and smoke plumes. The peak velocity of 410 mm s^{-1} occurs in the gaseous plume, 2 mm in front of the paper burn line. The velocities in the smoke plume are generally less than half of this peak velocity.

Measurements at different distances above the cigarette show that the plume is accelerating as it rises above the cigarette (Fig. 3). This acceleration is accompanied by a falling temperature of the plume. The falling temperature of the plume with distance above the cigarette is accomapanied by increasing density. This, along with the observed acceleration, means that there is a substantial increase in mass flow rate in the plume with distance above the plume. This is brought about by air being radially drawn into the buoyancy driven, natural convection sidestream plume as it moves upwards. Detailed calculations and mathematical modelling have confirmed that this occurs (Robinson 1987; Robinson 1988).

Also shown in Fig. 3 are two previous measurements of the velocity of the sidestream smoke plume, that of Neurath et al. (1966) and that of Ayer and Yeager (1982). Both their values were single point measurements, i.e., they did not show any variation of velocity with distance above the cigarette. Clearly the actual flow structure of the sidestream plume is more complex than these two earlier measurements imply.

In general the same chemicals present in mainstream smoke are also present in sidestream smoke, though their relative yield per cigarette is highly dependent on the compound considered. Some typical sidestream/mainstream ratios are shown in Table 1 (Baker 1981; Guerin 1987; Klus and Kuhn 1982; Sakuma et al. 1983, 1984a, 1984b; Norman et al. 1983; Umemura et al. 1986). These ratios were obtained from different studies using different tobaccos, cigarette types, methods of collecting the sidestream smoke, and air movement conditions around the cigarette. These differences will affect the results, reflected by the quoted range for a given compound in

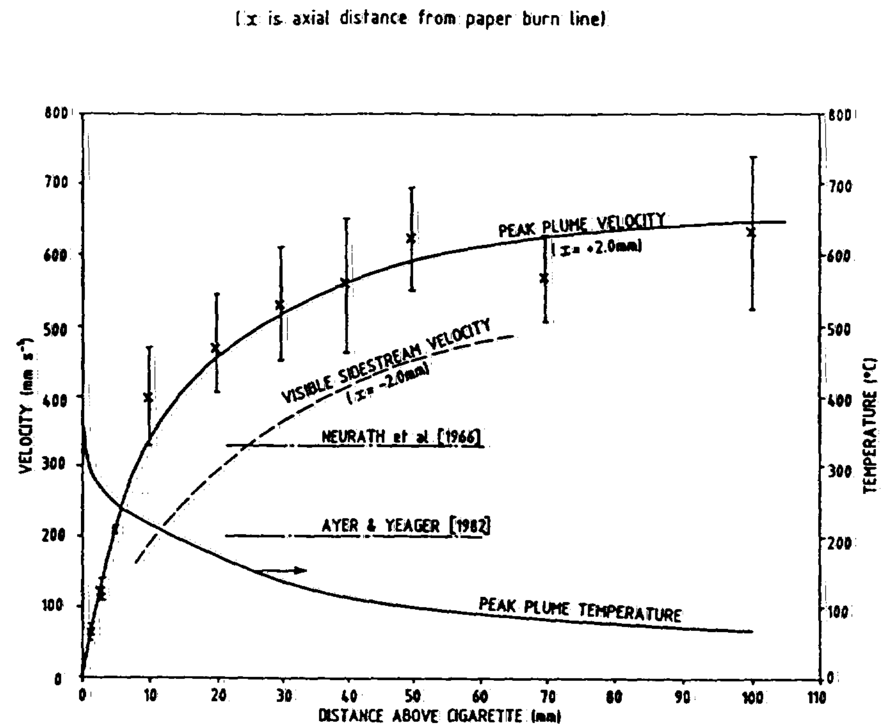

Fig. 3. Measured flow development in the sidestream plume (Robinson 1987).

Table 1. Some typical sidestream/mainstream (SS/MS) yield ratios.

Smoke Component	SS/MS
Hydrogen cyanide	0.06 - 0.5
Succinic acid	0.4 - 0.6
Hydroquinone	0.7 - 1.0
Neophytadiene	1.1 - 1.8
Phenol	1.6 - 3.0
Nicotine	1.9 - 3.3
Acetic acid	1.9 - 3.9
Carbon monoxide	2.5 - 4.7
Benzo(a)pyrene	2.7 - 3.4
Nitric oxide	3 - 13
Limonene	4 - 12
Toluene	5.6
Carbon dioxide	8 - 11
Acrolein	8 - 13
Pyrrole	9 - 14
Naphthalene	17
Pyridine	10 - 20
Water	30
Ammonia	44 - 170
Nitrogen	>270

the table. However, the data do show that there is a very wide range of ratios, varying from 0.06 - 0.5 for hydrogen cyanide to over 270 for molecular nitrogen formed chemically from the tobacco. Of course, one to four times more tobacco is burnt in smoulder than during puffing, depending on the cigarette parameters. Thus, some of the ratios in Table 1 (e.g., phenol and nicotine), simply reflect the proportions of tobacco burnt to the two smoke streams. However, there are many substances which distribute themselves so predominantly to one stream or the other that the reason cannot be due to differences in tobacco consumption. The reasons lie in the different conditions of temperature and mass transfer rates existing in the cigarette burning zone during smoulder and puffing, and the exact mechanism by which different components are formed or released from the tobacco.

During a puff, air is drawn into the peripheral regions of the burning zone, solid phase temperatures in excess of 900°C are reached and it is largely the periphery of the cigarette that burns (Baker 1981). When the puff ceases, the periphery of the burning

zone cools rapidly to about 600°C, air is converted into the back of the burning zone and the axial portion of the tobacco rod is preferentially consumed during about the first twenty seconds of smoulder. The temperatures in the centre of the burning zone are in the region of 800°C and only increase by 50 - 80°C as the puff progresses. The major combustion products, carbon dioxide, carbon monoxide and water, are formed in the high temperature (> 500°C) region of the burning zone. However, the vast majority of smoke species are formed by pyrolysis/distillation processes in a relatively low temperature (< 500°C) oxygen deficient region, just behind the combustion zone in the region of the paper burn line. The concentrated organic vapour so formed is drawn down the tobacco rod during the puff to the mainstream and largely diffuses radially out of the rod during smoulder to form the sidestream smoke.

Hydrogen cyanide is formed via decomposition of nitrates and amino acids. The predominance in the mainstream must reflect a high temperature formation mechanism with sufficient temperatures hardly being attained during smoulder. On the other hand, ammonia, which is formed from the reduction of nitrates and pyrolysis of glycine, is delivered predominantly to the sidestream. Sufficient temperatures must exist during the smoulder period for the pyrolytic generation of the ammonia. Vapour phase water is also delivered almost exclusively to the sidestream and is believed to be derived from oxygen reacting with pyrolytically-generated hydrogen as it diffuses into the sidestream plume (Johnson 1975). Thus, the exact ratio in Table 1 very much depends on the mechanistic origin of each component.

The pH of mainstream and sidestream smoke also differs, with sidestream smoke being generally more alkaline. For example, the pH of the mainstream smoke of a U.S. blended cigarette is typically in the range 6.0 to 6.2 and that of the sidestream is in the range 6.7 to 7.5 (Brunnemann and Hoffmann 1974). In this study, the pH was determined by passing smoke over a sensitive combination electrode connected to a pH meter. The observed sidestream and mainstream difference are due to the predominance of basic components in the sidestream, e.g., ammonia, pyridine and nicotine, and carboxylic acids and phenols in the mainstream smoke. (However, pH is a concept applicable to aqueous solutions and strictly speaking it is not meaningful to give too much significance to the pH of a suspension of aerosol particles).

The mean size of the aerosol particles in sidestream smoke is smaller than in mainstream smoke. Thus, Okada et al. (1977), using a light scattering technique, reported mainstream particles to [illegible] geometric number mean diameter of [illegible] µm and sidestream particles of 0.12 µm. The mean size of the mainstream particles is smaller than that reported in other studies using other measurement techniques, cited by Okada et al. (1977). However, the relative size distributions of the mainstream and sidestream particles are seemingly authentic. The different size distributions for the two smoke streams must reflect the different rates of cooling and levels of air dilution to which their precursor vapours are subjected.

In mainstream smoke, Browne and co-workers (1980), have shown that nicotine is almost entirely in the particulate phase. This also is true for fresh, concentrated sidestream smoke (Proctor 1988a), though nicotine seems to transfer rapidly to the vapour phase as the smoke stream ages and becomes diluted (Eudy et al. 1986; Eatough et al. 1986, 1989).

Sidestream smoke yields will be dependant upon the weight of tobacco burnt during smoking, the construction of the cigarette, and the way in which it is smoked. However, typically, a conventional cigarette will yield around 600 mg of CO_2, 4.5 mg CO, 5 mg of nicotine, and 25 mg particulate matter (water and nicotine subtracted) per cigarette in sidestream smoke.

Exhaled mainstream smoke

Few authors have considered exhaled mainstream smoke (EMS) as anything other than a minor contributor to ETS (Nystrom and Green 1986). Work recently completed in our laboratory suggests that the role of exhaled mainstream smoke should be considered in more detail. A variety of smokers were studied under carefully controlled conditions, smoking three types of cigarettes. These were a typical filtered British flue-cured cigarette (15 mg mainstream particulate delivery), a typical filtered case flavoured U.S. blended cigarette (14 mg), and a low delivery (3 mg) filter ventilated flue-cured cigarette. For each experiment three subjects smoked one cigarette type in their normal manner in a 30 m^3 chamber. This was repeated on three occasions for each of the cigarette types. For each experiment the maximum concentrations were determined for particulate matter (as measured by a piezobalance, TSI 5000), nicotine (Tenax trapping, GC-MS analysis) and carbon monoxide (by non-dispersive infrared spectroscopy).

Subsequently, cigarettes were smoked by machine (set at average parameters to mimic the human smoking) with mainstream smoke exhausted from the room. Hence the ETS from sidestream smoke alone was

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Table 2. Mean contributions of exhaled mainstream smoke (EMS) to ETS.

Cigarette	Contribution of EMS (%) to		
	ETS Carbon Monoxide	ETS Particulate Phase	ETS Nicotine
Flue-cured	11	43	7
US-blended	13	15	9
Filter Ventilated	3	20	1

compared to ETS produced by humans, including exhaled mainstream smoke.

Table 2 presents the summary data for the three cigarette types. It can be seen that exhaled mainstream smoke contributes little to the gas phase of ETS and that this contribution is dependant upon the mainstream delivery of the cigarettes studied. However, EMS does significantly contribute to the ETS particulate phase. It is thought that particulate matter is retained by the smoker to a greater extent than carbon monoxide, and hence it would be expected that the EMS particulate contribution would be less than observed (Creighton 1973). The results may then indicate that EMS particles are more stable (perhaps larger and containing more water) than fresh sidestream particles.

AGEING OF TOBACCO SMOKE

As sidestream and exhaled mainstream smoke diffuse into the atmosphere and away from the cigarette and smoker, they become ETS. The originally concentrated sidestream and exhaled mainstream smoke streams become greatly diluted, the sidestream smoke cools and accelerates (Figs. 1, 3), and various physical and chemical changes occur in the smoke.

A variety of studies (Eatough et al. 1986, 1987; Eudy et al. 1986; Hammond et al. 1987) have shown that the nicotine in ETS is almost entirely in the vapour phase. Since ETS nicotine originates almost entirely from sidestream smoke (see Table 2), then nicotine in the fresh sidestream particles must rapidly evaporate out of the particles as the smoke ages during initial dilution.

Studies by Pritchard and co-workers (Black et al. 1987; Pritchard et al. 1988) have also shown that matter is evaporated from fresh sidestream particles as they are diluted to form ETS. They loaded 1-iodohexadecane labelled with ^{123}I onto cigarettes. This material has a boiling point of 380°C, typical of that of components found in smoke particles. When fresh sidestream smoke was collected from the smouldering, loaded cigarette using a "fish-tail" chimney collection system described elsewhere (Proctor et al. 1988a) it was found that 5% of the sidestream radioactivity was found in the vapour phase and 95% in the particulate phase. On the other hand when the cigarette smouldered in a steel chamber of 14 m^3 internal volume, 70% of the airborne radioactivity was found to be in the vapour phase, and subsequent radiochemical analysis indicated that there had been no chemical degradation of the 1-iodohexadecane in the environmental chamber. Thus, the material had evaporated out of the sidestream particles during dilution to form ETS.

Ingebrethsen et al. (1985, 1986) have independently estimated that 20 to 30% of the original matter in sidestream particles is lost by evaporation during the ageing of ETS. This estimate was calculated from measurements of the number and sizes of sidestream particles diluted in a stirred 0.5 m^3 stainless steel chamber. The size distribution measurements were made by Ingebrethsen and Sears (1985) using a combination of an optical particle counter, an electrostatic mobility analyser, and a condensation nucleus counter. Sidestream smoke was introduced into the 0.5 m^3 chamber and diluted, and the number and sizes of the particles monitored over six hours. The estimated initial mass loss by evaporation of the smoke particles during about the first hour of ageing resulted in ETS particles with a mean number diameter of 0.098 µm at typical ETS concentrations (several $\mu g\ m^{-3}$ particulate matter). This is equivalent to a mass median diameter of 0.185 µm. As the ETS aged over the next few hours in the stirred chamber, there was a slow but gradual increase in mean mass median diameter: 20% increase over 12 hours. This is due to a combination of coagulation of particles and removal of smaller particles by deposition onto surfaces of the chamber. Surface deposition of ETS is the main route of removal in a static environment and

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is a function of particle size, mixing rate, room size, and shape.

In addition to physical changes, chemical changes also occur as ETS ages. Thus, for example, nitric oxide slowly oxidises over minutes and hours to nitrogen dioxide in ETS (Piadé and Fink 1987; Klus et al. 1987; Baker et al. 1988). However, these chemical changes, and indeed the particle size changes described above, will be far outweighed by the physical effects of air movement which will occur in real indoor environments. These effects are described in the next section.

BUILD-UP AND DECAY OF ETS CONSTITUENTS

As cigarettes are smoked in a room, the levels of ETS components in the room rise and then fall due to air circulation, room ventilation and, to a lesser extent, interactions of the ETS constituents such as deposition of smoke particles onto surfaces in the room. It is normally not practical to measure the dynamic build-up and decay of ETS constituents in real-life environments and for such information specially constructed environmental rooms are used. In such rooms, the temperature, relative humidity, air circulation rate, and fresh air input can be varied over a wide working range and the observed dynamic ETS levels related to room environmental conditions. A number of studies in such rooms has been published in recent years (Hoegg 1972; Cain and Leaderer 1981; Case 1985; Blake et al. 1986; Heavner et al. 1986; Ingebrethsen et al. 1986; Black et al. 1987; Eatough et al. 1987; Hiller et al. 1987; Olander et al. 1987; Piadé and Fink 1987; Pritchard et al. 1988; Rawbone et al. 1987a, 1987b; Vu Duc and Huynh 1987; Baker et al. 1988) or in standard offices with controlled environments (Klus et al. 1987).

Typical results from one study (Baker et al. 1988) are illustrated in Fig. 4. In this study the ETS was produced from the sidestream smoke of nonventilated filter cigarettes containing flue-cured tobacco smoked under standard smoking machine conditions in a 30 m^3 chamber. The chamber walls and ceiling had an impervious painted plastic finish and the floor was constructed of heavy duty non-slip PVC. Further details of the chamber have been given elsewhere (Case 1985; Baker 1988). Room conditions and number of cigarettes smoked were varied over the following ranges: temperature, 15-30°C; relative humidity, 55-85%; room air circulation, 1-27 air changes per hour; fresh air, 8-25%; number of cigarettes, 2-8.

During an experiment, samples of air were continuously removed from the room and analysed for particulate matter (using a TSI Model 5000 piezobalance fitted with a 3.5 μm filter situated in the room), carbon monoxide and carbon dioxide (using non-dispersive infrared spectroscopy), nitric oxide, and nitrogen dioxide (using chemiluminescence), hydrocarbons (using flame ionisation), and particle size and number distributions (LAS-X laser aerosol spectrometer). For nicotine determination, time weighted average values were obtained over the five minute periods by sampling the air through thermal desorption tubes containing Tenax and subsequent analysis by gas chromatography/mass spectrometry. Room temperature and relative humidity were also monitored during each experiment. The data from all the instruments were monitored on a microcomputer, taking data points every 30 seconds. All results presented are corrected for background readings.

Individual data points are included on some of the profiles in Fig. 4 to indicate the precision of measurement. The signal to noise ratio for most of the instruments was better than 20:1, although that for nitrogen dioxide and total hydrocarbons was 5:1 and 10:1 respectively, due to the analysers being operated at maximum sensitivity. The coefficients of variation of all the profiles over five replicate experiments were better than 5%.

In Figs. 4 and 5, gas concentrations are quoted in mg m^{-3} or μg m^{-3}. It is, however, common practice in studies on ETS to use the units 'parts by volume per million' (ppm), which is equivalent to μL/L in the metric system of units, especially for carbon monoxide levels — see, for example, the review by Repace (1987b). The exact conversion factor depends on gas density, and for carbon monoxide at room temperature and pressure, 1 mg m^{-3} = 1.15 ppm.

All the measured ETS components in Fig. 4 reach a maximum concentration at the end of smoking and then decay exponentially at a first order rate, i.e., decay rate is proportional to component concentration, as indicated by linear $\log_e$ concentration/time plots (not illustrated). The nicotine profile apparently reaches a plateau after it has decayed to about 20% of its maximum value. The profiles for particle number concentration and aerosol mass are almost exactly parallel, as are the profiles for carbon monoxide and carbon dioxide. The count median diameter of the ETS aerosol particles remained constant at 0.13 μm as the ETS built up and decayed over the 5000 seconds of the determination. This is larger than that reported by Ingebrethsen and Sears (1985) in their 0.5 m^3 chamber, due to the laser aerosol spectrometer in the current study not measuring the small particles below 0.1 μm which were included in In-

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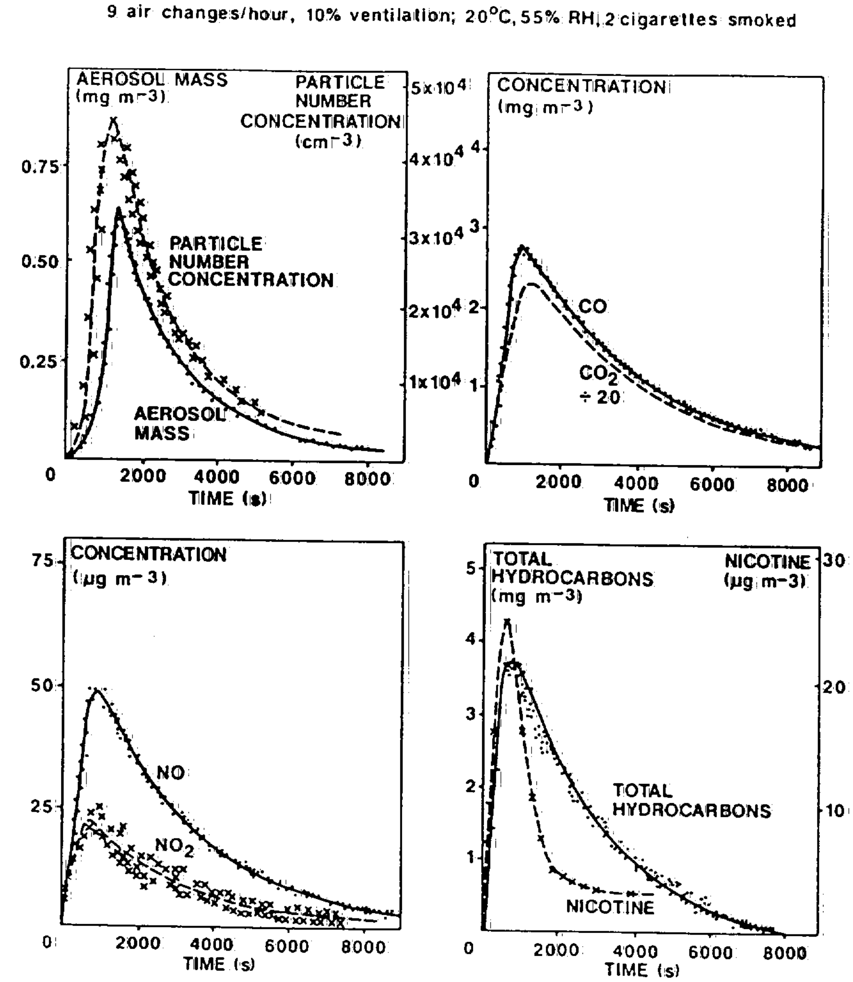

Fig. 4. ETS build-up and decay profiles.

gebrethsen and Sears' combined technique described earlier.

For all the components studied, the peak ETS concentration (or time-weighted average for nicotine) increases linearly with the number of cigarettes smoked for 2 to 8 cigarettes. This linear relationship has been observed previously with up to 30 cigarettes smouldered (Hoegg 1972; Case 1985; Blake et al. 1986). Using two types of experimental cigarettes with filter ventilation levels of 20 and 50%, Blake and co-workers (1986) have demonstrated the linear relationship for the following ETS components: carbon monoxide, nitric oxide, nitrogen dioxide, hydrogen cyanide, ammonia, formaldehyde, phenol, each of the three cresols, nicotine and aerosol particle mass.

The time taken for the peak ETS concentration to decay back to the background level depends on the environmental conditions. Fig. 5 illustrates the effect of air exchange rate (equal to room air circulation rate multiplied by the fraction of fresh air admitted to room) on the decay of carbon monoxide. Clearly, air movement has a large effect on the decay, as expected from mathematical considerations (Repace 1987a; Robinson 1988). The effect of air exchange rates on the half-life times of ETS components, obtained from profiles similar to those in Figs. 4 and 5 is illustrated in Fig. 6 over the sixteen sets of environmental conditions used in the study.

Half-life time is defined as the time to decay to half the value of the maximum concentration. Half-

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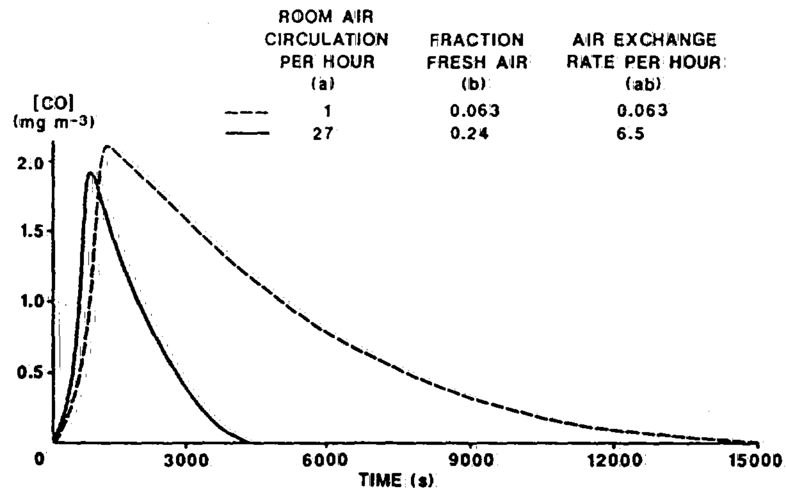

Fig. 5. ETS CO profiles for two room conditions.

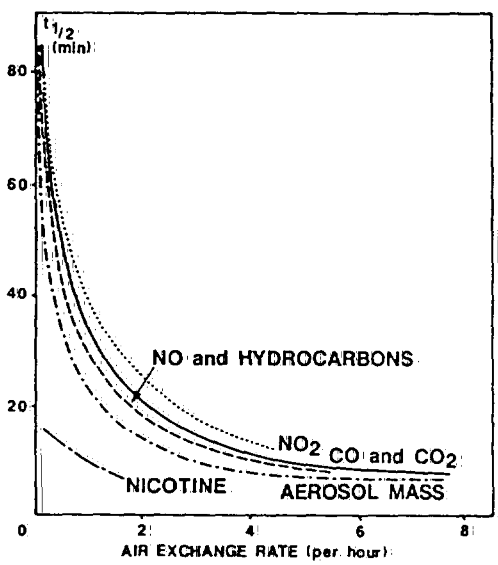

Fig. 6. Variation of ETS $t_{1/2}$ values with air exchange rate.

life times for nicotine were not obtained under all the conditions in the study, and in general are much smaller than the values for the other components, and much less influenced by the air movement. They are also less accurately defined, since they are based on average levels over five-minute periods rather than continuous measurements, and since nicotine is decaying relatively fast. Similar $t_{1/2}$ values for nicotine can be obtained from the decay profiles reported in other studies: values of 10, 14 and 23 minutes from Eudy and co-workers' profiles (1986) and 16 and 23 minutes from the profiles of Rawbone and co-workers (1987a). Variation of the environmental temperature (15-30°C) and relative humidity (55-85%) had a negligible effect on the $t_{1/2}$ values (detailed results not illustrated).

For a given set of environmental conditions, the trends in Fig. 6 indicate that the relative $t_{1/2}$ values of the ETS components are in the order:

$$NO_2 > CO = CO_2 > NO \approx \text{total hydrocarbons} > \text{aerosol mass} > \text{nicotine}$$

The decay rates of the carbon oxides depend solely on the effects of air change rate and fresh air input (Piadé and Fink 1987) and their levels in ETS remain constant when there is no air movement (Heavner et al. 1986; Hiller et al. 1987). Where a component has smaller $t_{1/2}$ values than carbon monoxide there must be some mechanism other than just air movement depleting its levels — chemical or physical. Nitric oxide $tv_{1/2}$ values are on average significantly less than those for carbon monoxide, and nitrogen dioxide $t_{1/2}$ values are significantly higher, at the 90% confidence level. This is due to the conversion of nitric oxide to nitrogen dioxide in the ambient air, as postulated by others (e.g., Piadé and Fink 1987; Klus et al. 1987).

$$2NO + O_2 \rightarrow 2NO_2 \qquad [1]$$

In fact, using the experimental data on ETS nitric oxide and nitrogen dioxide decay levels it can be shown that the rate constant for reaction [1] in ETS

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is almost an order of magnitude higher than that for the pure gas phase conversion but similar to that in the gas phase of mainstream smoke (Baker et al. 1988). Thus, in ETS, and the gas phase of mainstream smoke, the oxidation of nitric oxide is catalysed by substances which are not known.

The smaller values of $t_{1/2}$ for aerosol mass than carbon monoxide must be due to deposition of the particles onto the surfaces in the room. The very much smaller values for nicotine could be due to adsorption of nicotine vapour onto the surfaces — since, as indicated in the preceding section, nicotine in ETS is largely in the vapour phase.

REAL-LIFE LEVELS OF ETS COMPONENTS

Although ETS originates from sidestream and exhaled mainstream smoke, the great dilution and other changes which these smoke streams undergo as they form ETS make their properties significantly different from those of ETS. Thus, the sidestream/mainstream ratios quoted in Table 1 can be misleading if used out of context. The important question is not the ratio of sidestream/mainstream but rather what is the concentration of the constituent in the indoor environment and how does it compare to levels from sources other than ETS. Studies based solely on observations of fresh sidestream, or highly and unrealistically concentrated ETS, should take into account the possible differences between these smokes and ETS found in real-life situations.

The previous sections have described how the concentration of ETS in a room will depend upon many factors such as the number of cigarettes smoked, how they were smoked and what type they were, and on the size and ventilation conditions in the room. The situation is further complicated in the real world by the fact that ETS is only one contributor to indoor air containing chemicals arising from multiple sources (Proctor et al. 1988b). All indoor air environments contain numerous chemicals as a result of emissions from, for example, building materials, furnishings, cooking and heating fuels, and consumer products (NRC 1981). Many of the chemicals associated with ETS will also be present as a result of such sources (Jenkins and Guerin 1984; Proctor et al. 1988a, 1989a, 1989b).

Hence, in order to determine potential exposures to ETS, it is essential to employ methods that allow a distinction between substances present as a result of tobacco smoking and substances present as a result of the other various sources. One approach is to identify a chemical marker that is specific, or at least indicative, to ETS (Haley et al. 1988).

Much early research used carbon monoxide concentrations to assess ETS levels (Sterling and Dimi 1982). However, there are many sources of carbon monoxide, such as gas cookers or heaters, open fires, or motor vehicle emissions drawn in from outdoors, and it is not possible in real-life situations to segregate the ETS contribution from this background (NRC 1981; Girman and Traynor 1983; Haley et al. 1988).

Nicotine is far more specific, and had been used in recent years. The presence of nicotine in air is almost certainly indicative that tobacco smoking is, or has been taking place. Typically indoor levels range from 5 to 70 $\mu g\ m^{-3}$.

However, it should be noted that the behaviour of nicotine in ETS is somewhat unusual when compared to many of the other constituents. Nicotine is primarily a vapour phase constituent in ETS, though a small portion (around 2%) will be found associated with the particulate phase (Eatough et al. 1989). Research has shown that the nicotine decays rapidly from an atmosphere in comparison even to other vapour phase constituents of ETS (Nystrom and Green 1986; Eatough et al. 1989 and Fig. 4 of the present paper). Moreover, it is also likely that some nicotine adsorbed onto walls and furnishings will be re-emitted. If this were to be the case, then areas where smoking had not taken place for some time might still exhibit a low level of airborne nicotine.

Even so, nicotine is currently the most useful specific chemical marker for ETS, and many field studies have utilised its measurement. For example, Thompson et al. (1989) found airborne nicotine concentrations in restaurants ranged from 0.5 to 37.2 $\mu g\ m^{-3}$ with a geometric mean of 3.5 $\mu g\ m^{-3}$. In offices, Hammond et al. (1987) found personal exposures to nicotine (nonsmokers) ranging from 3.1 to 28.2 $\mu g\ m^{-3}$, whilst Carson and Erikson, (1988) using fixed site monitoring, in a study of 31 offices in Ottawa found airborne nicotine geometric mean to be 7.2 $\mu g\ m^{-3}$ (range <1.2 to 69.7 $\mu g\ m^{-3}$). Research undertaken by our laboratory found similar levels of airborne nicotine with a median of 3.1 $\mu g\ m^{-3}$ (range 0.6 to 26 $\mu g\ m^{-3}$) in smokers' offices, 15.5 $\mu g\ m^{-3}$ (range 0.6 to 49.3 $\mu g\ m^{-3}$) in smoking-allowed train compartments, and 18 $\mu g\ m^{-3}$ (range 3 to 57 $\mu g\ m^{-3}$) in betting shops (Proctor et al. 1989a, 1989b).

Respirable suspended particulates (RSP) have also been determined in indoor air in relation to ETS. Virtually all ETS particulate matter will be in the respirable fraction (i.e., less than 3.5 μm diameter) of airborne particulate matter.

However, in real-world situations, ETS will seldom be the sole source of particulate matter. Hence it is important to try and estimate the proportion of particulate matter relating directly to ETS, rather than just measuring total particulate matter. One of the most frequently referenced papers is the work of Repace and Lowrey (1985). Their research suggested that a typical nonsmoker working in an office building in the U.S. would be exposed daily to average concentrations of particulate matter due specifically to ETS of 242 $\mu g\ m^{-3}$ (range 100 to 1000 $\mu g\ m^{-3}$). However, Samet et al. (1987) stated that surveys of indoor air quality based on measurement of total suspended particulate concentrations (such, presumably, as the Repace and Lowrey study) will not readily identify the excess mass indoors from environmental tobacco smoke.

Some researchers have attempted to develop more specific methodologies for the determination of the ETS proportion of total RSP. One method extracts respirable particulate matter collected on teflon coated filter pads, and subsequently analyses the extracts for their ultra-violet (UV) absorbance at 325 nm. By using a surrogate standard, 2,2^1,4,4^1-tetrahydroxybenzophenone, calibrated against ETS formed in controlled conditions, an estimate of the ETS contribution to particulate matter can be made (Thomas et al. 1989). This measure has been termed UV-RSP, and will often be an over-estimate, as smoke of the particulate matter in indoor air originating from sources other than ETS will also result in UV absorbance.

An alternative method, based on the same philosophy, analyses the methanol extracts of collected particulate matter for fluorescence (Thomas et al. 1989). Sample solutions are calibrated against dilute mainstream tobacco smoke solutions, and the method is suggested to have a greater sensitivity and selectivity than the UV-RSP measure.

A more specific particulate marker may be the compound solanesol. This substance is present in relatively high levels in tobacco and is transferred intact to sidestream smoke (Ogden and Maiolo 1988). However, it should be noted that different types of tobacco contain different levels of solanesol and hence the ambient concentration will be dependant not only on the number of cigarettes smoked but also, to some extent, on which brand of cigarettes was smoked.

Relatively few studies have published data using these particulate partitioning methods. Our data from offices, train compartments, and betting shops (Proctor et al. 1989a, 1989b) suggest that, although ETS does add to the particulate levels in indoor environments, it may not always be the predominant source, and is unlikely to be the sole source. In smokers' offices a median RSP level of 91 $\mu g\ m^{-3}$ (range 33 to 260 $\mu g\ m^{-3}$) was found, though the corresponding UV-RSP data (the estimate of ETS contribution) gave a median value of 24 $\mu g\ m^{-3}$ (range 0.5 to 75 $\mu g\ m^{-3}$). This would suggest that in a relatively well ventilated office environment, ETS was contributing approximately, on average, 26% to the total RSP level. In smoking allowed-train compartments, a median RSP level of 249 $\mu g\ m^{-3}$ (range 71 to 325 $\mu g\ m^{-3}$) was identified. The corresponding UV-RSP data here were a median of 70 $\mu g\ m^{-3}$ (range 13 to 110 $\mu g\ m^{-3}$). This would suggest an approximate 28% contribution of ETS to total particulate matter. In betting shops (smoking allowed) the median RSP was 284 $\mu g\ m^{-3}$ (range 73 to 767 $\mu g\ m^{-3}$) whilst the median UV-RSP was 109 $\mu g\ m^{-3}$ (range 57 to 610 $\mu g\ m^{-3}$); an approximate ETS contribution of 38%. This research suggests the necessity for future studies to attempt to apportion ETS particulate matter from total particulate matter.

Although ETS contains many chemicals, research has been unable to identify good chemical markers apart from nicotine and ETS-specific RSP measures. Eatough et al. (1989) have suggested that 3-ethenylpyridine, myosamine, nitrous acid and pyridine may be possible markers for ETS gas phase, though more research is necessary to determine the validity of these substances.

An alternative approach to determining the contribution of ETS to an environment is to compare directly smoking and nonsmoking situations. In order to do this successfully, it is essential to match closely the factors impacting on the environments; for example smoking and nonsmoking situations should be of similar size, ventilation conditions, occupancy, furnishings, etc. This may be achieved by selecting sites within the same building, or by taking large numbers of sites for a particular environmental category. For example, Spengler et al. (1981) measured total RSP values in 80 homes to conclude that a smoker of 20 cigarettes per day would contribute 20 $\mu g\ m^{-3}$ to 24-hour indoor particulate concentrations. This type of approach is most usefully undertaken using ETS specific markers, but it may also identify differences in the levels of nonspecific chemicals associated with ETS. In the U.S. Environmental Protection Agency's Total Exposure Assessment Methodology (TEAM) study, levels of some airborne volatile organic chemicals (VOC) were suggested to be higher in smokers' homes than in nonsmokers' homes (Wallace et al. 1987). Other small studies have been unable to dis-

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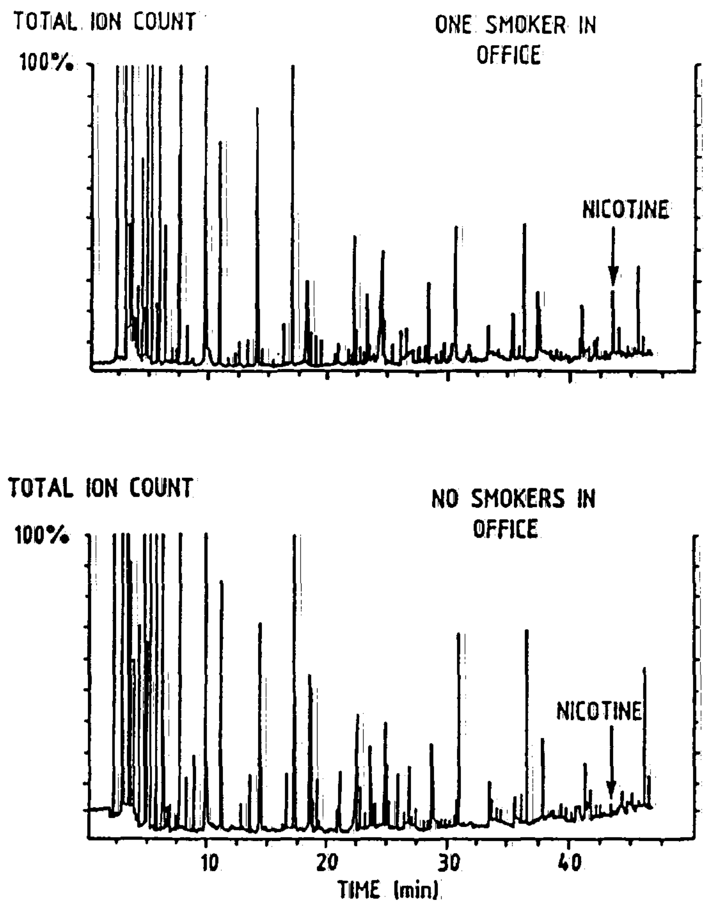

Fig. 7. Chromatographic profiles of the air in a smokers' and a nonsmokers' office in a modern, air-conditioned building.

tinguish between the VOC levels in smokers' and nonsmokers' offices (Bayer and Black 1987; Proctor et al. 1989b).

Fig. 7 illustrates a comparison of volatile chemicals in the air of a smoker's and a nonsmokers' office in the same building. The chromatographic profiles were acquired by drawing air through a sampling tube containing Tenax TA adsorbent for an hour sampling period. VOCs are trapped on the Tenax, and recovered for analysis by subsequent thermal desorption — capillary gas chromatography — mass spectrometry (Proctor et al. 1988b). The two offices were virtually identical, apart from the presence of one smoker (who smoked 3 cigarettes during the sampling period), and three nonsmokers in what is termed the smoker's office, and the presence of four nonsmokers in the other office. The nicotine peak in the smoker's office corresponded to a level of 6 $\mu g\ m^{-3}$. Apart from nicotine, a detailed analysis, using mass spectrometry revealed a similar range of volatile organic compounds (such as benzene and styrene) and similar concentrations of these chemicals in both the smoker's and nonsmokers' office. This finding is not surprising, as the nicotine peak would have to dominate the chromatographic profile in order that the VOC contribution from ETS could be detected above the background level (nicotine being the predominant ETS-associated volatile). A small amount of nicotine (0.5 $\mu g\ m^{-3}$) was found in the nonsmokers' office, but the similarity in VOC levels between smoking and nonsmoking situations is not due to recirculation of chemicals from smoker's offices because both UV-RSP and nicotine levels in the nonsmoking areas were far lower (by a factor of at least 10) than in smoking areas.

Hence, the accurate assessment of ETS in real-life situations relies upon the use of specific chemical markers to distinguish the ETS contribution from the

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chemical background, arising from various sources, that is present whether smoking is taking place or not.

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THE CHEMICAL CHARACTERIZATION OF ENVIRONMENTAL TOBACCO SMOKE

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ABSTRACT

A detailed characterization of environmental tobacco smoke is needed in order to understand at least four areas which are important to the proper evaluation of the impact of smoking on the nonsmoker in indoor environments: 1. identification of the chemical compounds to which the nonsmoker is exposed, 2. determination of changes in the chemical composition and in the gas-particulate phase distribution of environmental tobacco smoke with time in the indoor environment, 3. identification of substances which may be used to estimate the exposure of the nonsmoker to environmental tobacco smoke in the indoor environment, and 4. risk assessment of disease and irritant exacerbations associated with exposure of the nonsmoker to environmental tobacco smoke. The first three areas are reviewed in this paper.

THE CHEMICAL COMPOSITION OF FRESH ENVIRONMENTAL TOBACCO SMOKE.

Environmental tobacco smoke consists of sidestream smoke generated by a burning cigarette and mainstream smoke exhaled by the smoker. The major contribution to environmental tobacco smoke is from the sidestream smoke from a cigarette. Sidestream and mainstream smoke produced during the smoking of a cigarette are chemically qualitatively similar. However, significant quantitative differences exist between the two sources. In addition, as the generated environmental tobacco smoke emissions are introduced into an indoor environment substantial changes in the gas-particulate phase distribution of the chemical species present occurs.

Chemical Composition of Sidestream Tobacco Smoke.

The chemical characterization of environmental tobacco smoke has mainly been limited to the comparison of the concentrations of organic (1-7) and inorganic (8,9) constituents in particles of tobacco smoke condensate and total sidestream tobacco smoke, the determination of the main inorganic gases present in environmental tobacco smoke such as CO_2, CO, NH_3, HCN, NO and HNO_2 (1-3,10-12), and the determination of the principal organic compounds present in environmental tobacco smoke in chamber studies (13-19) and in indoor environments where environmental tobacco smoke is present (15,20-27).

The chemical composition of environmental tobacco smoke has been extensively discussed in reviews by the Surgeon General (1) and the National Research council (2). More recent data have been discussed in recent reviews (3,28-30) and in a recent IARC Monograph (31,32). Included in the IARC Monograph and other publications is a review of the combustion conditions associated with the formation of both mainstream and sidestream tobacco smoke (3,33). The major differences between mainstream and sidestream tobacco smoke arises from the lower combustion temperatures associated with the formation of sidestream tobacco smoke (3,33), leading to an increase in the amounts of distillation products and a decrease in the amounts of combustion products present. As a result, sidestream smoke is more alkaline than mainstream smoke (34). This alkalinity results from the increased amounts of N-containing bases present as distillation products in the sidestream smoke aerosol. However, the combustion zone of mainstream smoke is more oxygen deficient than the combustion zone of sidestream smoke and, as a result, the CO_2/CO ratios are higher for sidestream smoke than for mainstream smoke.

The expected composition of freshly generated sidestream tobacco smoke is given in Table I The amounts of most constituents in sidestream tobacco smoke are little effected by brand, tobacco moisture content, or mainstream combustion parameters for combustion of comparable amounts of tobacco (3). As a result, sidestream tobacco smoke emissions, and since sidestream tobacco smoke is responsible for most of the environmental tobacco smoke in indoor environments, environmental tobacco smoke composition in an indoor environment should be predictable from a knowledge of the number of cigarettes smoked as a function of time. This prediction will be altered by changes in the phase distribution and chemical composition of the environmental tobacco smoke as it ages in an indoor environment.

Gas-particulate Phase Distribution of Some Compounds in Environmental Tobacco Smoke.

Little is known about the distribution of organic compounds between the gas and particulate phases of environmental tobacco smoke (2,3,6,13-15,35). Recent studies have shown that most of the volatile nitrogen containing compounds such as nicotine, myosmine and pyridine are predominantly present in the gas phase of environmental tobacco smoke (14-16,18,20,35,36). Acrolein, formaldehyde and acetaldehyde are major toxic constituents of the gas phase of environmental tobacco smoke (1,2). The distribution of many mutagenic and/or toxic compounds, such as the N-nitrosamines, between the gas and particulate phases of environmental tobacco smoke is presently not known (2). For most of the semi-volatile organic compounds the phase of the compound is not well known. The phase distribution of nicotine has been extensively studied. While nicotine is undoubtedly present primarily in the particulate phase in sidestream tobacco smoke generated in small combustion devices (3), it is present as the gas phase species in sidestream tobacco smoke in a chamber (37,38). The equilibration from the particle to the gas phase is very rapid (14,16,39,40). The more alkaline pH of sidestream tobacco smoke (34) may be, in part, responsible for

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Table I. Gas and Particulate Phase Compounds Potentially Useful as Tracers of Environmental Tobacco. (Data are From References 14,15,19,21,41,50,96)

Chemical Class	Class wt% of Particle	Examples of Identified Compounds	μMol Compound/Mol CO Gas Phase	μMol Compound/Mol CO Particles	% Compound in Gas Phase
Particles				4.22±0.82g/mol CO	
Mutagenicity				6.47±1.15revertants/μmolCO	
Alkanes/ Alkenes	3.6±0.5	Phytadiene	-----[a]	138± 13	-----
		n-Hentriacontane	-----	93± 29	-----
		1,3-Butadiene	3100±850	-----	100
		Isoprene	19200±5600	-----	100
		Solanesol	-----	219±43	0
Bases	10.9±1.2	Nicotine	12500±3600	393±150	98.3±1.4
		Myosmine	375± 38	14.7±1.7	96.1±0.9
		Nicotyrine	16± 6	10.6±3.3	60 ±20
		Cotinine	18.2±9.4	17.7±7.0	54.6±12.8
		Pyridine	1180± 72	< 5	100
		3-Ethenylpyridine	1370±460	< 5	100
		2-Ethenylpyridine	207± 36	< 5	100
Sterols	0.5±0.1	Campesterol	-----	6.4±2.4	0
		Stigmasterol	-----	12.1±6.5	0
		β-Sitosterol	-----	9.1±7.5	0
		Cholesterol	-----	5.9±1.3	0
Sterenes[b]	0.4±0.3	24-MeC-3,5-dien	-----	8.8±8.5	0
		24-EtC-3,5,22-trien	-----	6.8±3.4	0
PAH	<0.1	Pyrene	-----	0.02±0.01	-----
		Phenanthrene	-----	0.11±0.02	-----
Inorganic	-----	NO	36700±3200	0	100
		NO_2	2520± 430	0	100
		HNO_3	96± 127	92± 55	44 ±24
		HNO_2	4650±1300	54± 40	98.7± 1.0
		SO_2 + Sulfate	71± 52	71± 40	49 ±23
		NH_3	51900±5400	155± 34	99.6± 0.2
		Potassium	0	840±150	0
		Calcium	0	340±100	0

[a] ----- = not determined.
[b] MeC and EtC = Methylcholesta and Ethylcholesta.

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the rapid displacement of the non-protonated nicotine from the particles (3). In addition to nicotine, nicotyrine, myosmine, pyridine and alkyl pyridine compounds are also present only or predominantly in the gas phase (14,15,20, 39). The identification of the gas-particle phase distribution of organic compounds of toxicological interest is an area of needed research.

There is an apparent difference in total mass in sidestream emissions from a cigarette in studies conducted using a small combustion and collection device as compared to studies conducted using an environmental chamber (29). The chamber studies give emission values which range from 6 to 11 mg particles/cigarette while the other studies give values with 11 mg/cigarette as the lower end of the measurements. The differences cannot be accounted for based on selective deposition loss of particles in the chamber experiments as the data are generally obtained in chambers immediately after combustion of the cigarette (13,14,17,19). The difference appears to be real. Many of the organic constituents of sidestream tobacco smoke are volatile and losses of material from the particles as the smoke is diluted would be expected (41). It is assumed in the data interpretation given in this review that this is the case. The amount of particulate matter in environmental tobacco smoke resulting from the combustion of a cigarette is assumed to be about 10 mg.

If the differences in particulate matter observed in the two types of chemical characterization experiments (chamber vs combustion apparatus) are due to volatilization of organic compounds as the smoke is diluted into a room, then available data (29) suggest that about 1/2 of the generated sidestream particle mass is rapidly lost to the gas phase during dilution and aging in an indoor environment. Gas phase hydrocarbon measurements (14) and the results of radiotracer labeled experiments (42) are consistent with this hypothesis. The existence of large amounts of gas phase hydrocarbons which can be readily condensed back to particulate phase material has also been suggested by chamber experiments involving UV radiation of sidestream environmental tobacco smoke (14). The concentration of particles present in the irradiated sidestream mixture doubles in less than one hour as a result of chemistry induced by the UV radiation. This includes the movement of most of the gas phase nicotine back to the particulate state (13,14). Comparable chemistry apparently occurs in environments with unvented combustion heaters. The gas phase compounds responsible for this observed chemistry are not now known. The identification of gas phase compounds in environmental tobacco smoke may help determine which species are responsible for sensory irritation in sensitive individuals (43-46).

CHANGES DURING THE AGING OF ENVIRONMENTAL TOBACCO SMOKE CONSTITUENTS IN AN INDOOR ENVIRONMENT.

Environmental tobacco smoke is a complex mixture of gas and particulate phase compounds. During aging of environmental tobacco smoke in an indoor environment, changes in the chemical composition will occur. These changes will include coagulation of particles to alter the particle size distribution (13,47,48) changes in the gas/particle distribution of semi-volatile compounds (14), and possible chemical changes due to reactions (13,14,22,39,40,49). In addition, the chemical composition of environmental tobacco smoke may be altered during aging in an indoor environment because of differences in the removal rate of various constituents as the environmental tobacco smoke is aged, is recirculated in the indoor environment and is mixed with outside air (22,23,35,50).

Many gas phase components of environmental tobacco smoke have been shown to be rapidly removed in indoor environments. Several studies have shown that the removal rate for gas phase nicotine and other basic nitrogen compounds is much faster than the removal rates for particles or non-reactive gases such as CO

(15,18,19,22,23,28,35,39,40,50). The relative removal rates may depend on local environmental factors such as wall coverings, furnishings, presence or absence of people, air flow, etc. Thus, environmental tobacco smoke will be a constantly changing mixture due to loss of material as a result of adsorption or decomposition and due to changes in gas/particulate phase equilibria for volatile species.

Several studies in controlled indoor environments have shown that nicotine is removed much faster than are CO, RSP, NO, gas phase hydrocarbons or specific particulate phase compounds (18,22,23,50,51). The importance of these laboratory observations with respect to environmental tobacco smoke in typical indoor environments is best illustrated by comparing the concentrations of nicotine to fine particulate matter in both controlled and indoor atmospheres. The ratio of RSP to total nicotine has been reported by several investigators. Experiments conducted in chambers with inert walls or in experiments with minimal residence time of the generated sidestream smoke (14,17-19,50,52) give a ratio of about 2-4 g RSP/g nicotine in diluted side stream tobacco smoke. Values determined in sidestream smoke emissions vary from 2 to 6 g RSP/g nicotine (3,4,7,11) with the higher values probably resulting from partial loss of some nicotine to the gas phase during sample collection on a filter (37,38,52). The studies of environmental tobacco smoke conducted in non-Teflon chambers give higher ratios of RSP to nicotine, probably because of the more rapid removal of gas phase nicotine by chamber components (15,18,22,23,50,52). Results from decay studies in chamber or controlled environment experiments are shown in Figure 1. The ratio of nicotine/RSP increases slightly with time in experiments conducted in a Teflon chamber (13,14) because gas phase nicotine is stable in this environment, but the concentration of particles decreases by evaporation and by loss of particles to the chamber walls. In all the other study environments, the rate of decay of gas phase nicotine is greater than the rate of loss of particles.

Steady-state experiments in ventilated chambers with individuals present or in well controlled experimental indoor environments give values of around 10 to 15 g RSP/g nicotine (19,22,23,53-56). This ratio is larger than that which would be seen if people (and the accompanying absorptive surfaces) were not present. For example, in studies in the chamber at the U.S. Environmental Protection Agency (19) the ratio of RSP to nicotine when the chamber had people in it was 13 g RSP/g nicotine. In the empty chamber, the ratio was determined to be 3.0 g RSP/g nicotine, a ratio consistent with the value obtained for sidestream smoke (3,5,7,11) and in inert chambers (13,14,50,52). It has been suggested that nicotine may be a good tracer of environmental tobacco smoke particles in indoor environments. However, experiments conducted in indoor environments indicate that the observed ratio of particles to nicotine present in atmospheres dominated by smoking varies from 3 to 80 g RSP/g nicotine (7,15,50,53,57-61), Figure 2. The ratio generally increases with increased residence time and/or decreased total nicotine concentrations. This same trend is observed for the limited data which are available on concentrations of nicotine and concentrations of UV-PM, which is believed to be indicative of the RSP originating from environmental tobacco smoke (28,66). Because of the rapid removal of nicotine from indoor environments, the use of nicotine as a tracer and of nicotine and its metabolites, cotinine and 3-hydroxycotinine, as biological markers for determining exposure to environmental tobacco smoke in studies of passive smoking has been questioned (15,28,29,50). The measurement of gas phase nicotine or even total nicotine may underestimate exposure to the particulate phase and possibly other components of environmental tobacco smoke.

There are a few studies which report changes in the chemical composition of environmental tobacco smoke as it ages in an indoor environment. In the absence of external oxidizing agents or UV light, NO in environmental tobacco smoke is only very slowly converted to NO_2, HNO_2, and possible organic nitrogen-oxide compounds (13). In the presence of a strong UV light source, this conversion is rapid (13). In addition, under these extreme conditions, the photochemistry

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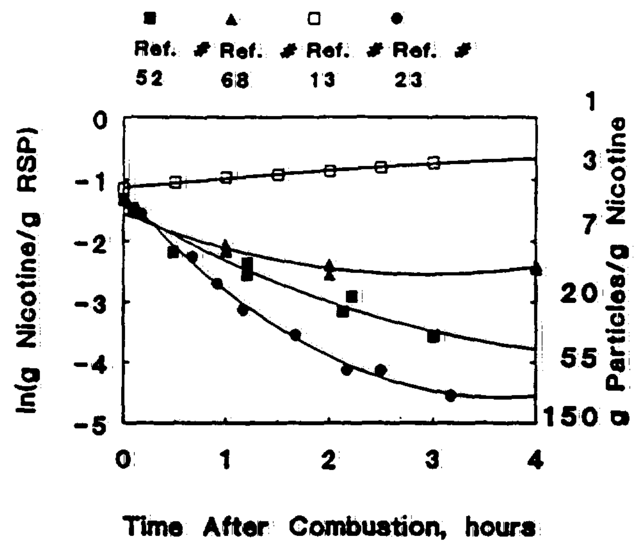

Figure 1 Change in the ratio of nicotine to RSP with time in chamber and controlled indoor experiments (Data are from reference 28).

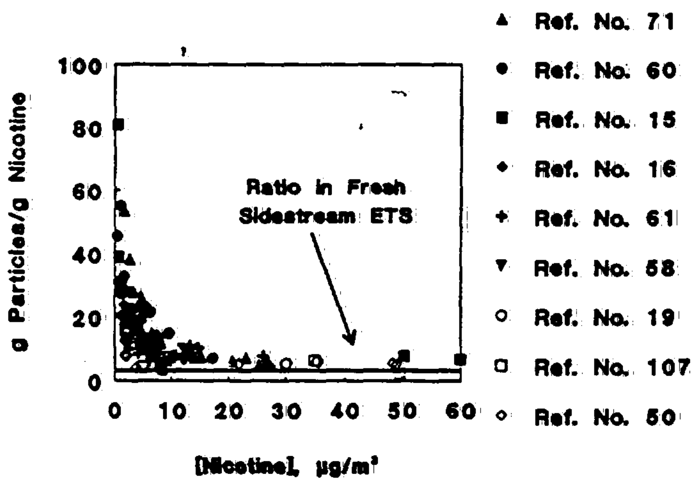

Figure 2 The ratio of RSP to nicotine as a function of nicotine concentration in indoor environments. The concentration of nicotine on the abscissa is divided by 2 for the open data points (from reference 28 and 50).

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leads to the doubling of total particulate matter, the reduction of gas phase basic compounds, loss of solanesol and photochemical alterations in the N-containing bases of environmental tobacco smoke (13,39,40,49). The exact chemical reactions taking place are not known. Under more realistic conditions where some oxidants are introduced into an indoor environment from the ambient air (22), the same reactions appear to occur but at a greatly reduced rate.

These changes in environmental tobacco smoke composition over time complicate the assessment of health effects associated with exposure, and may preclude the accurate measurement of human exposure to specific compounds without actual measurement of the compounds of interest.

TRACERS OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE

The assessment of environmental tobacco smoke exposure is complicated by the presence of significant amounts of organic material in both the gas and the particulate phase and by changes in the relative composition of the two phases with time. The development of accurate markers for estimating exposure to environmental tobacco smoke will be dependent on the determination of the chemical composition of both the gaseous and particulate components of environmental tobacco smoke and the elucidation of changes in that composition with time in indoor environments. As summarized in the National Academy of Sciences review (2), a suitable tracer for quantifying environmental tobacco smoke exposure should be:

- unique or nearly unique to environmental tobacco smoke.
- easily detected in air, even at low smoking rates.
- similar in emission rate for a variety of tobaccos.
- in constant proportion to compounds in ETS which affect human health.

One of the major shortcomings of many studies is that only one measure of exposure to environmental tobacco smoke was determined. Therefore, there is no way to assess whether the concentration of the species studied is (or is not) related to the components in environmental tobacco smoke which may be hazardous to health.

Chemical tracers of environmental tobacco smoke used to assess exposure in the past have included RSP, CO, NO_x and nicotine. Recent reviews of environmental tobacco smoke studies by the National Academy of Sciences (2) and the U.S. Surgeon General (1) reach the same conclusion: the only tracers previously used which may be related to actual exposure to environmental tobacco smoke are concentrations of nicotine and RSP.

<u>CO and NO_x as Tracers of Environmental Tobacco Smoke.</u>

Components of environmental tobacco smoke which are not specific to ETS but hich have been used to assess exposure to environmental tobacco smoke include :O (1,25,46,57,63,64) and NO_x (1,46,56). Both of these gases can be measured in ndoor environments by a variety of techniques as illustrated in the referenced ırticles. Comparison of the chemical composition of environmental tobacco smoke n chamber experiments, Table II, (13,14,17,18,64,65) and the relationship etween CO concentrations and other constituents of environmental tobacco smoke n indoor environments suggests that the majority of the CO in indoor nvironments comes from sources other than environmental tobacco smoke. Chamber xperiments show the ratio of CO to RSP in environmental tobacco smoke is about .2 to 0.4 mol CO/g RSP, Table I and II, (14,17,64,65). The ratio of CO to RSP n restaurants impacted by modest levels of smoking has been reported to average bout 4 (57) and 1.0 (66), and in a variety of homes and offices where smoking :curred at modest levels the ration of CO to RSP varies from 0.4 to 6 and rerages 2 (14). Even in environments such as taverns, discos, bus terminals ıd restaurants, where the major source of particulate matter is from smoking,

the CO to RSP ratio varies from 0.4 to 1.5 and averages 0.7 mol CO/g RSP (15,64,65) which is generally higher than seen in controlled chamber experiments. Measurements in restaurants of CO and UV-PM (a measure of particulate matter more specific to ETS, see next section) give a ratio of CO to UV-PM of 1.7 mol CO/g RSP (66), a ratio about five times that expected from environmental tobacco smoke. The recent study of environmental tobacco smoke in restaurants (66) included the determination of CO in the outdoor environment. The outdoor CO concentrations averaged about half of the indoor CO concentrations. Correcting the indoor restaurant data for the measured outdoor concentrations gives a minimum ratio of non-ambient CO to UV-PM of 0.9 mol CO/g UV-PM, about three times the expected ratio. Clearly, the majority of the CO in indoor environments comes from sources other than environmental tobacco smoke. Similarly, the ratio of NO_x to RSP reported in indoor environments varies from values comparable to that found in environmental tobacco smoke to ratios which are higher by factors of up to 10 (10). In addition, NO and NO_x have been shown to be only weakly correlated with CO from environmental tobacco smoke, even in a controlled indoor environment (56). The results available to date show that CO and NO_x concentrations will significantly overestimate exposure to environmental tobacco smoke (28).

Nicotine.

The only tobacco specific compound which has been used extensively in the past to determine exposure to environmental tobacco smoke is nicotine (15,19,53,54,57,58,61,67-69). It might expected that determination of the concentration of nicotine in an indoor environment would give a good measure of exposure to environmental tobacco smoke. The use of nicotine as a tracer of environmental tobacco smoke is complicated because nicotine is found primarily in the gas phase (18,20,36-38,53,54,57) and because gaseous nicotine is removed at a faster rate than particulate phase nicotine or the particulate portion of ETS (15,20,22,23,50,57). Thus the concentration of gas phase nicotine underestimates exposure to the particulate phase of environmental tobacco smoke and possible to the concentration of many gas phase environmental tobacco smoke constituents, e.g. see Figure 2 and related discussion in text.

Estimation of exposure to environmental tobacco smoke based on the determination of nicotine has been done both by determining airborne concentrations of nicotine and also by determining the concentration of nicotine and its principal metabolite, cotinine, in body fluids (68,70-88). Studies in which exposure has been determined by the use of questionnaires, and dose measured by urine levels of nicotine and cotinine (72,75,76-79,81,82,84,85,89) have generally yielded poor correlations between exposure and dose. In contrast, studies which have determined urine clearance of nicotine and/or cotinine and have also directly measured nicotine (68,76,77) or ETS (90) exposure have yielded better correlations when the measurements were made over time intervals for exposure which are long compared to the rate of clearance of the compounds from the body (71,74,91-94). However, it is now known that determination of exposure to ETS by measurement of gas phase (or total) nicotine probably underestimates exposure to many components of environmental tobacco smoke in most indoor environments by as much as an order of magnitude. This is due to the rapid removal of gas phase nicotine as compared to many other constituents of environmental tobacco smoke (2,10,15,20,22,23,28,29,50,52,57). The usefulness of nicotine and cotinine as biomarkers of exposure to constituents of environmental tobacco smoke is limited by the viability of nicotine itself as a surrogate for environmental tobacco smoke.

Respirable Particulate Matter.

Total RSP is the tracer for environmental tobacco smoke most extensively used in past studies (1,2) because of the ease with which it may be measured. Even though RSP is elevated in environments where smoking is present, about one half of the RSP in these indoor environments comes from sources other than

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environmental tobacco smoke. RSP thus overestimates exposure to environmental tobacco smoke (15,25,66,96). The measurement will be even more insensitive for the determination of ETS exposure as controls are implemented and the concentration of tobacco smoke decreases in controlled environments.

A technique which has the promise of being specific for particulate matter from environmental tobacco smoke (47,62,66,101) is based on the measurement of the UV absorption of a methanol extract of the collected particulate matter. The resulting measure is referred to as UV-PM, UV determined particulate matter. The UV-PM measurement is better correlated with the estimated amount of environmental tobacco smoke than is the corresponding measurement of nicotine (28,62,66). The measurement of UV-PM may provide a specific measure of particulate matter from only environmental tobacco smoke (62,66,101) but additional work needs to be done to determine if other sources of RSP may contribute to the UV-PM concentrations.

Particulate Mutagens.

Studies of particulate mutagens in indoor atmospheres suggest that environmental tobacco smoke may be the principal source of mutagenic compounds in particles present in indoor atmospheres (55,59,68,98,99). While there are many potential sources of mutagens in the environment (100-103), current data suggest that environmental tobacco smoke is the principal source of particulate mutagens in indoor environments (19,55,99) and that the emission rate of mutagenic material in the particulate phase of environmental tobacco smoke is constant (65,99). The mutagenic activity of particulate matter in rooms where smoking is occurring is much higher than concentrations seen in the absence of smoking, and the mutagenic activity is correlated with the number of cigarettes smoked (55) and is consistent with the results of chamber experiments (19). However, the determination of particulate mutagens in indoor environments requires the use of specialized microsuspension techniques (55,65).

McCurdy, et al. (59) have reported on the simultaneous determination of the mutagenicity of particulate matter, concentrations of RSP and concentrations of nicotine in a bingo parlor and in a casino. A comparison of the observed concentrations of RSP and mutagens (28,59) indicates that in the environments studied there is a background of non-mutagenic particulate matter of about ** g/m^3 with increasing mutagenicity of the particulate matter as the concentration of environmental tobacco smoke RSP increases. The mutagenicity of this ETS-RSP agrees with the mutagenicity of ETS determined in chamber studies (65,99). The ta also indicate that nicotine from environmental tobacco smoke is lost more rapidly than either RSP or nicotine in the indoor environment and that the concentration of nicotine leads to an underestimation of environmental tobacco smoke particles and particulate mutagenicity (28). The loss of nicotine may also explain the lack of correlation of RSP mutagenicity with biological measures of exposure, i.e. urine concentrations of cotinine and nicotine in the population studied (59). Further studies on the potential use of mutagenicity as a measure of exposure to environmental tobacco smoke are needed. It should be noted that urine mutagenicity cannot be used to assess exposure (2,87, 105,106) because of the effect of other sources of mutagens, such as diet.

Other Potential Conservative Tracers of Environmental Tobacco Smoke.

The fourth criteria from the National Academy of Sciences review of environmental tobacco smoke (2) listed in the introduction to this section requires that a tracer for assessing exposure to environmental tobacco smoke be present in the indoor atmosphere in constant proportion to compounds in environmental tobacco smoke which affect human health. The tracer which has been emphasized in studies conducted to the present is gas phase nicotine. While gas phase nicotine meets the requirements of being unique to ETS, being easily measurable in the environment and having a similar emission rate for many commercial brands of cigarettes, the concentration of nicotine relative to most other constituents of environmental tobacco smoke is not constant in an indoor

environment. The initial data available on the two tracers, UV-PM (62,66) and particulate mutagens (71), indicate that these may be conserved relative to most components of environmental tobacco smoke in an indoor environment. It is not clear, however, that the measurement of these two tracers will be unique to environmental tobacco smoke, especially at low concentrations of emission and subsequent exposure to a population. In addition, neither of these two tracers can be used to develop techniques for the assessment of biomarkers for determining human dose. Additional tracers are needed.

The results from studies by Brigham Young University (15,22,23) and a larger study by R.J. Reynolds Tobacco Company (66) suggest that 3-ethenylpyridine may be a conservative tracer of environmental tobacco smoke in indoor environments. This compound is present at about concentration expected relative to environmental tobacco smoke particulate matter in a variety of indoor environments (15,22). In contrast, the relative amount of nicotine present in indoor environments is much lower than expected based on the chamber experiments because of the selective loss of gas phase nicotine. The potential use 3-ethenylpyridine as a tracer of RSP in environmental tobacco smoke should be studied further. 3-ethenylpyridine is present only in the gas phase in the indoor environment and may be determined using passive sampling devices (14), diffusion denuders (14,15) or sorbent beds (22,39,40,66). In fresh environmental tobacco smoke there is about 9 times as much nicotine as 3-ethenylpyridine, Table I. However. because of the selective loss of gas phase nicotine in indoor environments, the concentration of gas phase 3-ethenylpyridine in indoor environments is comparable to the concentration of gas phase nicotine (15,22). 3-Ethenylpyridine may be determined with a detection limit comparable to that for nicotine using similar sampling systems.

A compound unique to the particulate phase of environmental tobacco smoke which should be studied as a potential tracer is solanesol (21,49,66,104). Solanesol, (3,7,11,15,19,23,27,31,35-Nonamethyl-2,6,10,14,18,22,26,30,34-Hexatriacontanonaen-1-ol),

$$CH_3\text{-}\overset{\overset{CH_3}{\|}}{C}\text{—}CH\text{-}[CH_2\text{-}CH_2\text{-}\overset{\overset{CH_3}{|}}{C}\text{=}CH]_8\text{-}CH_2\text{-}OH,\ MW - 631$$

is a trisesquiterpenoid of trans isoprene units which is present at about 3 wt% in the particles from environmental tobacco smoke, Table I. The large molecular weight assures that it will be present only in the particulate phase in environmental tobacco smoke. The compound can be collected by conventional indoor sampling systems and determined by gas chromatography (21,49,56,104), or supercritical fluid chromatography (49). The limited data available to date (21,49,66,104) indicate that the concentration of solanesol in environmental tobacco smoke particles is conserved in the environment.

A recent report by Löfroth et al. (19) of chamber studies of environmental tobacco smoke suggests that isoprene may be used as a gas phase tobacco smoke tracer in indoor environments. The amount of isoprene emitted in sidestream smoke is comparable to the amount of nicotine produced, Table I. In addition, samples collected indoors and outdoors of a tavern indicate that background concentrations of isoprene may be negligibly small compared to the indoor concentration from environmental tobacco smoke. Equally important, the ratio of isoprene to RSP, nicotine or particulate mutagenicity from environmental tobacco smoke in the tavern was comparable to that observed in the chamber studies. Additional work needs to be done in indoor environments with lower concentrations of environmental tobacco smoke to determine if isoprene is a conservative tracer and to establish if other sources, such as vegetation, may be significant.

Gas phase 3-ethenylpyridine and isoprene, and particulate phase solanesol may be good tracers for environmental tobacco smoke (15,21-23,65,66,104).

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However, the viability of these compounds as tracers for assessing exposure to environmental tobacco smoke has not yet been extensively tested (15,66). The relationships among these compounds and between these compounds and other constituents of environmental tobacco smoke in indoor environments needs to be established. If 3-ethenylpyridine and/or isoprene are shown to be conservative tracers for both many gas phase constituents and for the particulate phase of environmental tobacco smoke, then these could possibly serve as the basis for a tobacco specific passive monitor or for a biomarker for assessing exposure to environmental tobacco smoke.

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Mainstream and Environmental Tobacco Smoke

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Environmental tobacco smoke (ETS) is derived from cigarette smoldering and active smoker exhalation. Its composition displays broad quantitative differences and redistributions between gas and respirable suspended particulate (RSP) phases when compared with the mainstream smoke (MSS) that smokers puff. This is because of different generation conditions and because ETS is diluted and ages vastly more than MSS. Such differences prevent a direct comparison of MSS and ETS and their biologic activities. However, even assuming similarities on an equal mass basis, ETS-RSP inhaled doses are estimated to be between 10,000- and 100,000-fold less than estimated average MSS-RSP doses for active smokers. Differences in effective gas phase doses are expected to be of similar magnitude. Thus the average person exposed to ETS would retain an annual dose analogous to the active MSS smoking of considerably less than one cigarette dispersed over a 1-year period. By contrast, consistent epidemiologic data indicate that active smoking of some 4–5 cigarettes *per day* may not be associated with a significantly increased risk of lung cancer. Similar indications also obtain for cardiovascular and respiratory diseases. Since average doses of ETS to nonsmoking subjects in epidemiologic studies are several thousand times less than this reported intake level, the marginal relative risks of lung cancer and other diseases attributed to ETS in some epidemiologic studies are likely to be statistical artifacts, derived from unaccounted confounders and unavoidable bias. © 1991 Academic Press, Inc.

INTRODUCTION

During the last decade, considerable attention has been devoted to the question of whether environmental tobacco smoke (ETS) causes disease in nonsmokers (USSG, 1986; NRC, 1986; EPA, 1990a). Some epidemiologic studies of nonsmokers presumably exposed to ETS have suggested a marginal increase of risk for some diseases previously associated with active mainstream smoking (MSS). These reported risks, however, border on statistical and epidemiologic insignificance, and could easily derive from numerous and documented biases and confounders.

Official reviews have stopped short of implying a causal role of ETS in most of these associations, with a notable exception for lung cancer. This exception has been based not so much on admittedly questionable epidemiology, but on a public health stance of concern driven by perceived—but largely undocumented—compositional similar-

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ities of ETS and MSS. and by the implausible assumption that no dose exists below which risks are nonexistent or imponderable (USSG, 1986; NRC, 1986; IARC, 1987; EPA, 1990a,b).

This study analyzes the scientific literature on the chemical and physical characteristics of MSS and ETS, their reported specific biologic activities, and the mean relative doses of active MSS smokers and ETS-exposed nonsmokers under prevailing real life conditions, and finally considers the epidemiologic projections that these combined influences imply.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF MSS AND ETS

Mainstream smoke is what smokers generate and inhale. Cigarettes smoldering between puffs emit side stream smoke (SSS), which, along with smoke exhaled by active smokers (EMS), becomes environmental tobacco smoke after immediate and progressive dilution, and aging. Both MSS and ETS result from the combustion of the same substrate and lead to exposures to analogous but not necessarily the same components, and certainly in different proportions, under different physical conditions, and at very different doses.

MSS is generated and exists in the well-defined confines of the cigarette and the mouth and the respiratory system of smokers. It is formed under conditions of high relative humidity, results in particulates with mean aerodynamic diameter of about 0.7 μm (Hiller *et al.*, 1982), and is inhaled within a few seconds of its formation with little aging or intervening transformations. All this permits a rather precise definition of its chemical and physical nature (Dube and Green, 1982).

On the other hand, any characterization of ETS must recognize its unstable and variable nature. The immediate dilution of side stream smoke with air begins a chain of physical and chemical transformations that continuously alter the ensuing ETS as it ages over hours. Smoker-exhaled mainstream smoke also contributes to ETS a certain fraction of gases and the small respirable suspended particulates (RSP) that are not retained in the lungs of smokers (Baker and Proctor, 1990). Actual SSS itself is difficult to define because its composition has been shown to vary under different conditions of experimental generation (Brunnemann *et al.*, 1978; Eatough *et al.*, 1990). Moreover, SSS is not indicative of the physical and chemical changes that occur in ETS as it continuously dilutes and ages.

The experimental generation of SSS-ETS in laboratory chambers after the smoldering of a few cigarettes has provided some clues to the composition of ETS (Benner *et al.*, 1989; Eatough *et al.*, 1989; Ingebrethsen *et al.*, 1988; Pritchard *et al.*, 1988; Vu-Duc and Huynh, 1989; Ingebrethsen and Sears, 1985). Better experiments have been attempted in larger chambers where humidity, temperature, ventilation rates, and other variables could be controlled, also leading to a perception of the differential influence of these variables on ETS decay (Eatough *et al.*, 1990; Baker and Proctor, 1990; Tang *et al.*, 1988; Ingebrethsen *et al.*, 1988). However, such data still fail to represent actual life conditions in real environments with a multismoker presence. No comprehensive studies have been published so far under such natural settings. The few available reports have attempted to measure only selected chemicals, sampled over variable but generally short periods of time, making them of dubious relevance to situations of prolonged exposure (Proctor *et al.*, 1989a,b; Lofroth *et al.*, 1988; Carson and Erikson, 1988; Sterling *et al.*, 1988; Oldaker *et al.*, 1987; Stehlik *et al.*, 1982). In the future,

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more useful data should come from real-life ambient conditions with smokers being present, where new ETS is continuously generated as aging ETS decays, eventually establishing quasi-steady-state conditions and an "average" chemical and physical composition of ETS. Thus far, only nicotine and RSP concentrations have been measured with some appearance of accuracy under such conditions.

While several thousand MSS components have been identified in the literature, only about 100 components of fresh SSS and EMS have been measured. In addition, only fragmentary information is available for diluted ETS components, most of which appear to be present—if at all—at levels beyond analytical capabilities (Guerin *et al.*, 1987; NRC, 1986; USSG, 1986; EPA, 1990a). Today only a few specific and some general inferences are possible about the differences of MSS and ETS. SSS, from which ETS mainly is derived, is generated under conditions of better oxygenation and contains proportionally less carbon monoxide than MSS. It also contains fewer products of pyrolysis and distillation, and undergoes immediate cooling and vast dilution with air, with the result that ETS-RSP have considerably smaller size than MSS-RSP, with mean aerodynamic diameters on the order of 0.1 μm, or 100 to 200 times smaller in volume than MSS-RSP (Ingebrethsen *et al.*, 1988; Ingebrethsen and Sears, 1985). Smaller particles tend to evaporate faster and more efficiently than larger ones, so that many substances associated with MSS-RSP are more prevalent in the gas phase of ETS. This and the somewhat more alkaline conditions of SSS cause nicotine to appear in the ETS gas phase almost exclusively, while it is mainly associated with particulates in MSS.

As ETS ages, particle concentration and total mass decrease because smaller particles eventually coalesce, while mass is lost to diffusion/evaporation and to electrostatic and gravitational deposition (Hinds, 1978; Benner, 1989). The process continues with dynamics that depend on temperature, pressure and relative humidity, electrostatic conditions, ambient geometry and surface composition (furniture, fabrics, paints, crowding, etc.), ventilation rates, type and number of cigarettes smoked, mode of smoking, and other variables (Baker and Proctor, 1990).

In time, ETS gases and RSP adsorb to ambient surfaces and are disposed of by ventilation, while adsorbed substances may again desorb and recirculate in gas or vapor form, as has been suggested to happen with nicotine. Complex chemical transformations also occur because of interactions among molecules, oxidation, and probably photochemistry when UV radiation is present (Proctor, 1990). These continuous transformations occur at rates peculiar to each environmental situation and therefore result in physical states and chemical compositions that can be substantially different from place to place and from time to time.

In general, the better measures and estimates of ETS pertain to suspended particulates, a complex of substances that is apparently more stable and more measurable than individual substances. MSS-RSP appears to contain the smoke fractions capable of producing certain tumors in experimental animals. However, given that ETS and MSS have substantial differences in component concentration and partitions between gas and particulate phases, the issue of their relative biologic activity cannot be answered beyond some sensible conjecture. Past and also recent laboratory studies indicate that the biologic potencies of MSS-RSP and SSS-RSP seem virtually equivalent, with no detectable potency noted in the semivolatile fractions (Grimmer *et al.*, 1988; Stanton *et al.*, 1972). These data, however, pertain to the relative position of MSS-RSP and SSS-RSP condensates, and their relationship to ETS-RSP condensates has not been resolved. Other studies have reported that the *in vitro* mutagenic activity of MSS and

ETS may be roughly comparable, although the biologic significance of such data is speculative (Claxton *et al.*, 1989).

MEASUREMENT OF EXPOSURE

In the case of MSS the relative ratios of smoke components remain comparatively stable. Based on internal markers, the measurement of exposure and dose intake has been reasonably well defined, especially as pertains to RSP, nicotine, carbon monoxide, and other specific components (Gori, 1990). However, and despite several attempts at definition, selected markers have been rather disappointing surrogates for total ETS intake or exposure estimates.

A reasonable environmental marker should be specific to ETS, be easily detectable, and have a nearly constant ratio to other ETS components (NRC, 1986). Given the variable chemical and physical nature and the extreme dilution of most components of ETS, it is not surprising that a satisfactory marker of exposure has not been identified. For that and other reasons, an internal marker of actual ETS intake or dose has proven to be even more difficult to identify.

Most epidemiologic studies have measured exposure by means of recall questionnaires, with results that are problematic even at qualitative levels. Aside from the inability of virtually all epidemiologic studies to define whether exposure or lack of it was correctly reported—especially with data from proxy respondents—the issues of intensity and duration of exposure have hardly been addressed by questionnaires. Even when problems of subject misclassification, respondent bias, and correction for background ETS exposure could be addressed, questionnaires have produced no more than rough indexes of exposure. The collection of dependable information on actual doses at specific target sites and at different times has not been possible (Wu-Williams and Samet, 1990; Cummings *et al.*, 1989; McCarthy *et al.*, 1987).

Hopes have been placed on nicotine and its metabolite cotinine as possible markers of ETS intake and actual internal dose (Cummings *et al.*, 1990; Jarvis, 1989; Coultas *et al.*, 1987; Jarvis *et al.*, 1985; Hoffmann *et al.*, 1984). Unfortunately ETS-nicotine resides mostly in the gas phase and decays at rates quite different from other ETS components, to which it will have ratios that are variable in time and largely unpredictable (Tang *et al.*, 1988). Plasma cotinine levels suffer from similar and other shortcomings, although they have been shown to correlate with self-reported exposure to ETS (Cummings *et al.*, 1990; Jarvis, 1989). Reports also suggest that physiologic clearance of nicotine and cotinine at low plasma levels may proceed at much slower rates, likely because of slower release from preferential body compartments (Lewis *et al.*, 1990). Until these low-level kinetics are better understood, low plasma levels of nicotine and cotinine are likely to lead to substantial overestimations of intake doses. As such, nicotine and cotinine may provide a dichotomous index of contemporary exposure, but they remain inadequate as quantitative estimators of exposure, actual ETS dose, or their variation over an individual's life.

The ratio of ETS-nicotine to ETS-RSP has been suggested as a possible means to determine RSP intake from plasma nicotine levels, but such ratios appear too variable to be useful (Oldaker *et al.*, 1989). DNA and protein adducts have been proposed as markers of internal ETS dose, even though their specificity to ETS and their relationship to disease, especially to cancer, are in question (Randerath *et al.*, 1989; Harris *et al.*, 1987). Moreover, recent studies have reported no increase in DNA adducts in non-

smokers exposed to ETS (Holz *et al.*, 1990). The reported mutagenicity of urine samples in ETS-exposed nonsmokers also has proven elusive as a marker, largely because results may not be distinguishable from background rates and because of interferences from dietary sources (Scherer *et al.*, 1987, 1989; Mohtashamipur *et al.*, 1987; Sorsa *et al.*, 1985).

There has been great interest in measuring ETS-RSP directly, since its physical properties make for more positive identification and because biologic activity may reside specifically in the particulate phase, both in terms of its components and in terms of its longer residence time and cellular contact in the lungs. However, suspended particulates in air may be derived from many sources, and measurements of ETS-RSP need to be corrected for non-ETS RSP background. Several studies have reported differential values in the same settings under smoking and nonsmoking conditions (Repace and Lowrey, 1980; Weber and Fisher, 1980; Sterling *et al.*, 1987; Miesner, 1988; Kirk *et al.*, 1988a,b; Turner, 1988). It is obviously difficult to duplicate conditions of room occupancy, clothing, human traffic, ventilation, etc., where the only changing variable is smoking or not smoking. These problems have been much discussed, so that greater credibility can be accorded to the latest published studies. According to the more recent measurements of ETS-RSP in homes and workplaces, and allowing for differences of nonsmoking and smoking situations, a liberal estimate of ETS-attributable-RSP mean concentration in ambient air is less than 50 $\mu g/m^3$ (Table 1).

In regard to exposure to ETS gas phase components, it is enlightening to compare the concentrations of representative ETS components with the corresponding threshold limit values (TLVs) as established by the American Conference of Governmental and Industrial Hygienists for workplace safety. Incidentally, such values include considerable safety factors and are usually lower than the permissible exposure levels (PELs) established by the National Institute for Occupational Safety and Health (NIOSH)

TABLE 1

CONCENTRATIONS OF RSP FROM ETS AND OTHER SOURCES IN VARIOUS ENVIRONMENTAL SETTINGS WITH AND WITHOUT SMOKER PRESENCE

		RSP concentration ($\mu g/m^3$)	
Reference	Site	No smoking	Smoking
Coultas *et al.* (1990a)	Homes	NA	17
Sheldon *et al.* (1989)	Homes	22[a]	65[a]
Spengler *et al.* (1981)	Homes	NA	20
Spengler *et al.* (1985)	Offices	39[b]	72[a]
Proctor *et al.* (1989b)	Offices	8[b]	23[b]
Oldaker *et al.* (1990)	Offices	NA	27[b]
Miesner (1988)	Offices	15[a]	36[a]
Sterling *et al.* (1983)	Office buildings	15[a]	29[a]
Coultas *et al.* (1990b)	Workplaces	NA	64[a]
Oldaker *et al.* (1990)	Restaurants	NA	36[b]
Crouse (1988)	Restaurants	NA	34[b]
Proctor (1990)	Public transport	14[b]	36[b]

Note. NA, data not available or not applicable.

[a] Based on total RSP.

[b] Based on UV-RSP portion of total RSP.

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and the Occupational Safety and Health Administration (OSHA). Table 2 gives some examples for selected indicator substances representative of related chemical families.

The estimates in Table 2 assume maximum recorded SSS emissions, no ventilation, no surface adsorption, and no intervening decay of any sort. However, official reports give estimates of the range of the ratios of MSS/ETS concentrations for selected components (NRC, 1986). These were found to vary as follows:

	MSS/ETS ratios		
Nicotine	57,333	to	7,200,000
Benzo[*a*]pyrene	68	to	40,740
Acrolein	1,500	to	20,833
Benzene	112	to	7,167
Acetone	240	to	2,000

Such values show the extremes of dilution that ETS has displayed under various conditions, and suggest that the number of cigarettes required to attain TLVs under realistic conditions would be orders of magnitude higher than the conservative estimates listed in Table 2.

ESTIMATING RELATIVE DOSES OF MSS AND ETS RSP

Although certain assumptions are necessary in estimating ETS-RSP doses, they are based on simple facts or on measurements that are fairly well verified. With this in

TABLE 2

ESTIMATED NUMBER OF CIGARETTES REQUIRED TO REACH TLV LEVELS FROM SSS EMISSION OF SELECTED CHEMICALS IN A SEALED AND UNVENTILATED 100-m³ ENCLOSURE

SSS component	SSS output[a] (mg/cigarette)	TLV[b] (mg/m³)	Cigarettes required
Methylchloride	0.88	10.30	1,170
Hydroquinone	0.16	2	1,250
Cadmium	0.0007	0.01	1,430
Acetaldehyde	1.26	180	1,430
Acetic acid	1.5	25	1,660
Nitrogen oxides	2.8	50	1,780
Formic acid	0.525	9.4	1,790
Pyridine	0.39	16	4,100
Phenol	0.25	19	7,600
Methylamine	0.1	13	13,000
Benzene	0.24	32	13,300
Catechol	0.14	23	16,500
Nickel	0.0025	1	40,000
Dimethylamine	0.036	18	50,000
Hydrazine	0.00009	0.13	145,000
Acetone	1	1780	178,000
Benzo[*a*]pyrene	0.00009	0.2[c]	222,000
2-Toluidine	0.003	9	300,000
Polonium 210	0.4 pCi	3 pCi/liter[d]	750,000
Toluene	0.000035	375	1,000,000

[a] Data from EPA (1990a), Table C-2, pp. C-19 and 20.
[b] Data from ACGIH (1990).
[c] Based on the TLV for coal tar pitch volatiles.
[d] EPA (1990c).

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mind, such estimates seem reasonably realistic and less affected by obvious judgmental considerations. Nevertheless, in comparing RSP doses from ETS and MSS exposures it also seems reasonable to present results in analog form rather than as precise point estimates, in recognition of possible uncertainties.

Assuming a prolonged daily exposure of 10 hr, a typical person would be exposed to a daily ETS-RSP dose of roughly 0.3–0.4 mg after breathing ambient air with a 50 $\mu g/m^3$ ETS-RSP concentration, at the rate of about 0.7 m^3/hr (Crawford-Brown, 1987). This is equivalent to over a 1000-fold reduction compared with the inhaled dose of the average mainstream smoker.[2]

However, an important difference relates to aerodynamic particle size, which tends to reduce lung retention of the smaller and probably less charged ETS-RSP particles (USSG, 1986). Indeed, available studies indicate an 80–90% efficiency of retention of mainstream smoke RSP in the lungs of smokers (Mitchell, 1962; Dalhamn *et al.*, 1969; Hinds *et al.*, 1983), while other studies show that only some 10% of RSP may be retained in the lungs of ETS-exposed subjects (Hiller *et al.*, 1982). This retention differential has been recognized by the EPA (EPA, 1990a), even though reliable studies suggest a substantially greater difference (McAughey *et al.*, 1989, 1990; Crawford and Eckerman, 1983). Therefore, based on mass alone, the average dose of ETS-RSP retained in the lung may be less than 1/10,000 of average MSS-RSP smoker doses.

RSP retention, however, does not equate with tissue or individual cell dose, which is the important issue given that cancer is thought to begin with cellular events. Among other things, cell dose will depend on quantity available per cell, proximity to cell surface, cell surface exposed, cell surface permeability, and duration of contact. Cellular dose is therefore modulated by mucociliary clearance and by the permeability of lung epithelium (Gerde *et al.*, 1991). In this regard, published data indicate that average mucociliary clearance is some threefold greater in nonsmokers than in smokers (Vastag *et al.*, 1985; Foster *et al.*, 1985), while average airway mucosal permeability appears to be about one third as great in nonsmokers as in smokers (Kennedy *et al.*, 1984), probably as the result of a thicker and more viscous mucous layer (Zayas *et al.*, 1990). Although these studies involve a relatively small number of subjects, they indicate that the effective cellular dose in ETS-exposed nonsmokers may be further reduced by close to 90% when compared with active smokers, due to clearance and permeability factors alone.

Together, these considerations suggest that the lung cell doses for average ETS-exposed nonsmokers are probably between 1/10,000 and 1/100,000 of equivalent cell doses for average mainstream active smokers. In practical terms, this implies an annual retained dose of tobacco smoke components equivalent to far less than the dose from the active smoking of one cigarette somehow evenly dispersed over a 1-year period (see Footnote 2).

MSS AND ETS: EPIDEMIOLOGIC COMPARISONS

An annual ETS retained dose equivalent to the active smoking of less than one cigarette over the course of 1 year may be compared with the MSS dose/response associations reported with various health conditions. The limit of statistical significance

[2] The average smoker of 30 cigarettes per day inhales some 30 mg of nicotine (Gori and Lynch, 1985). The sales-weighted average tar/nicotine ratio for the smoke of commercial cigarettes is 15–18 (FTC, 1985). Therefore the average smoker inhales about 500 mg of tar daily.

TABLE 3

MAXIMUM LEVELS OF DAILY CIGARETTE CONSUMPTION AT WHICH LUNG CANCER RISK IN MALE SMOKERS MAY NOT BE SIGNIFICANTLY INCREASED FROM THE RISK OF NONSMOKERS, BASED ON EPIDEMIOLOGIC DATA (SEE FOOTNOTE 3)

Reference	Maximum cigarettes/day
British doctors[a]	6.3
Swedish men[b]	3.9
ACS, 9 states[c]	5.4
ACS, 25 states[b]	0.9
U.S. veterans[c]	0.6
Canadian veterans[c]	1.6
Japanese men[b]	3.1
California men[c]	7.0

[a] From Doll and Peto (1978).
[b] From USSG (1979), pp. 5–13, Table 2.
[c] From USSG (1982), p. 38, Table 6.

for such associations would provide a reasonable index of comparison. For lung cancer, data from independent studies listed in U.S. Surgeon General Reports yield the results in Table 3, after analysis by standard analytical procedures.[3]

Official sources refer to the British doctors study as the most reliable set of data for dose/response analysis (EPA, 1990a). This prospective study appears to be adequately documented and offers a reasonably accurate tracing of subjects (Doll and Peto, 1978). Thus, data in Table 3 indicate that the active smoking of 4–5 cigarettes/day is not likely to be statistically associated with elevated lung cancer risk. This assessment, presented here without undue claim of precision, is in accord with other estimates (Wynder, 1991). It suggests that since ETS retained doses are several thousand times less than MSS doses from this level of cigarette smoking, they appear insufficient to generate elevated risks of lung cancer.

This conclusion is consistent with an increasing body of scientific opinion that MSS may act as a weak promoter rather than as an initiator, supporting the implication of no observable epidemiologic risk at low doses (Albert, 1989; Doll and Peto, 1981;

[3] Individual studies were analyzed separately. Generally, for each study, the relative risk associated with the number of cigarettes consumed daily was listed. The first step in the analysis was to fit the data to a function of the form

$$RR = A_0 + A_1X + A_2X^2,$$

where RR is the relative risk, A_0, A_1, and A_2 are coefficients calculated by the maximum likelihood method, and X is the daily cigarette consumption. Cigarette consumption data are usually expressed in intervals, e.g., 1–9. The midpoint or the mean value of each interval × 1.25 was used in the calculation, justified by studies which indicate an average 30% underreporting of daily cigarette smoking (Hatziandreu *et al.*, 1989; La Vecchia, 1986; Jackson and Beaglehole, 1985). The highest consumption values are generally reported as open-ended, e.g., 40+, and here the midpoint was set at the given value plus 10. For the British doctors study the actual mean values of the intervals were available (Doll and Peto, 1978). The nonsmoker reference data points (0.1) were also entered in the regressions. Although other functions were examined, graphic and statistical analysis shows that the quadratic function provides an exceptionally good fit to the data, with a corrected multiple coefficient of determination close to 1 in each case. Critical values of daily cigarette consumption were calculated as the values at which the lower bound of the 95% confidence interval of the estimated RR was unity.

TABLE 4

MAXIMUM LEVELS OF DAILY CIGARETTE CONSUMPTION AT WHICH RISK FOR CORONARY HEART DISEASE MORTALITY IN MALE SMOKERS MAY NOT BE SIGNIFICANTLY INCREASED FROM THE RISK OF NONSMOKERS, BASED ON EPIDEMIOLOGIC DATA (SEE FOOTNOTE 3)[a]

Reference	Maximum cigarettes/day
U.S. veterans	1.5
ACS. 9 states	2.5
Japanese men	4.0
Canadian veterans	4.5
British physicians	4.5
Swedish men	2.5
California men	3.0
Swiss physicians	3.0

[a] Epidemiologic data from USSG (1983), p. 118.

Doll, 1978). Moreover, epidemiologic studies of MSS categorize exposure by number of cigarettes smoked, report multiannual exposure durations, and provide some evidence of commensurate latency times preceding diagnosis. By contrast, most nonsmokers in households may be exposed to ETS for only a few hours a day, which would further tend to increase the distance between MSS and ETS doses.

The daily levels of cigarette consumption compatible with no significantly increased risk for other diseases associated with active smoking appear to be of the same order as for lung cancer. Tables 4 and 5 report the analogous estimates for cardiovascular and respiratory disease mortality, with the implication that retained doses of ETS are unlikely to be associated with significant risk elevations for such diseases as well.

CLOSING REMARKS

Ordinarily it is extremely difficult to demonstrate the effects of an agent at low dose levels. Rather, after an effect becomes apparent at high doses, the interpretation is

TABLE 5

MAXIMUM LEVELS OF DAILY CIGARETTE CONSUMPTION AT WHICH RISK FOR RESPIRATORY DISEASE MORTALITY IN MALE SMOKERS MAY NOT BE SIGNIFICANTLY INCREASED FROM THE RISK OF NONSMOKERS, BASED ON EPIDEMIOLOGIC DATA (SEE FOOTNOTE 3)[a]

Reference	Maximum cigarettes/day
Chronic bronchitis	
U.S. veterans	5.5
Canadian veterans	2.6
Emphysema	
U.S. veterans	2.2
Canadian veterans	2.7
California men	5.5
Bronchitis and emphysema	
British physicians	3.0
U.S. veterans	

[a] Epidemiologic data from USSG (1984), p. 202.

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made that some effect, however small, would obtain at lower levels. Using some dose/response model an estimate of the effects at low doses, often including some statistical confidence intervals, is attempted.

The difficulty with low level determinations is that often the results fail to be significantly different from the null reference. In other words, the confidence limits of the effect would likely include the null reference, and/or the actual estimate of the effect might even be in the direction of protection instead of harm. Indeed, for most substances there is some threshold of tolerance below which the organism can cope without suffering adverse effects. For that matter it is apparent that levels at or below threshold might actually be beneficial in the sense of inducing and stimulating resistance, a process known as hormesis. This is in fact the case for virtually all beneficial and even essential substances, which would produce adverse effects when administered at excessive doses.

The ETS of public health concern is what is presented to average nonsmokers under commonplace environmental conditions and not the exceptional examples that can be created in laboratories. With this stipulation, ETS is a very elusive entity, undergoing continuous transformations at extremes of dilution that make efforts to define its chemical, physical, and biologic characteristics highly difficult. While the components of MSS/SSS may also be present in ETS, it is also clear that with few exceptions they are undetectable by the most sophisticated analytical procedures.

Despite these rarefied dilutions, an ETS hazard has been presumed from a conjectural association with MSS (EPA, 1990a,b; USSG, 1986; NRC, 1986). Central to this conjecture is the presumption of an equivalent chemical and biologic activity of MSS and ETS, and of the absence of low doses below which risk would be null or intangible. However, current understanding of composition alone is not sufficient to compare activities among MSS, SSS, and ETS, and the actual testing in biological systems suffers for two main reasons: the need to utilize concentrated laboratory surrogates that may have little relevance to actual ETS, and the unresolved obstacles to interpreting high-dose-related animal or *in vitro* data in terms of equivalent human responses at extremely low doses.

With this in mind, and even assuming that the biologic activities of MSS and ETS are of similar order, the reality of the extreme dilution of ETS remains. In this regard we have noted that current regulations allow workplace exposures to many ETS gas phase constituents at concentrations between thousands of and a million times higher than can be expected from commonplace ETS.

We also offered evidence that if epidemiologic investigations of MSS and lung cancer had been confined to the effects of exposure to a few cigarettes daily, they would have failed to yield significant risk signals. Similar evidence has been shown to hold for other diseases associated with active MSS smoking. At the same time it is apparent that subjects included in ETS epidemiologic studies were probably exposed to equivalent MSS-RSP doses below even a single cigarette *per year*. Therefore, marginal RR values associated with ETS exposures should be imputed to biases, confounders, and other weaknesses of the investigations, and any judgment that ETS exposure leads to lung cancer and other diseases would flow from argument, not from credible data.

In fact, the majority of epidemiologic studies of ETS suffer from what appear to be irreparable deficiencies. Earlier on we discussed the failure of epidemiologic studies in general to define exposure to ETS in terms of duration and intensity in any satisfactory way. The additional difficulties arising from misclassification of smoking status or ETS exposure have been amply described in the literature. The erroneous classi-

fication of actual smokers or former smokers as nonsmokers would have serious consequences on epidemiologic results, especially because smokers tend to be married to smokers. The National Research Council Committee on Passive Smoking outlined the knowledge that would be necessary to assess the impact of classification bias, namely, the proportion of the sample that was misclassified, the proportion of male and female subjects, the proportion of married couples that have the same smoking habits, and the relative lung cancer risk of misclassified smokers and nonsmokers (NRC, 1986). Although it is self-evident that knowing all this would eliminate classification bias, these variables have not been measured or reported in studies and therefore are subject to conjectures and assumptions that, however educated, have led to very different assessments (NRC, 1986; Lee, 1987a). In this regard it is possible to state only that misclassification bias is difficult to assess but very probable, and its impact could be of sufficient magnitude to explain the marginal lung cancer RRs reported by some ETS studies (Lee, 1987b).

An even greater prejudice to the credibility of ETS epidemiologic studies derives from their failure to account and control for the possible confounding by many independent risk factors. For lung cancer, a selected list of these is given in Table 6.

Since many of the RRs in Table 4 are substantially larger than any reported for the association of lung cancer and ETS, even weak contributions by combinations of these confounders would be cumulative and could be more than sufficient to explain the marginal lung cancer risks that some epidemiologic studies of ETS have reported. In fact it is likely to be so, because these studies have not controlled for the factors of Table 4 in any meaningful or comprehensive way, while other investigations provide evidence that several of those risk factors cluster and selectively segregate in families with smokers (Subar *et al.*, 1990; Morabia and Wynder, 1990; Sidney *et al.*, 1989; Whichelow *et al.*, 1988, 1991; Koo *et al.*, 1988; Pisani *et al.*, 1986; Friedman *et al.*, 1983). For cardiovascular diseases the independent risk factors reported in the literature number over 200, many of which are the same as apparent lung cancer risks (Hopkins and Williams, 1981). For respiratory diseases analogous independent risk factors have been identified, ranging from genetic to sociologic, to dietary and environmental conditions, also likely to cluster in households with smokers (Shilling *et al.*, 1977; Comstock *et al.*, 1981; Morris *et al.*, 1990; Schwartz and Weiss, 1990).

It should be clear that the seemingly insurmountable difficulties in measuring ETS exposures and doses, unresolved classification bias, and the inability to control for numerous independent confounders explain the inconsistency of weak ETS epidemiologic results and speak against scientifically credible conclusions about a risk that, if real at all, remains imponderable.

Indeed, the only justifiable conclusion is that this issue cannot be resolved scientifically on the basis of currently available information. Moreover, exposure and dose considerations alone seem to indicate that ETS is an insignificant entity among the substantial mass of exogenous and endogenous challenges to health that we continually face.

Hypothetical risks from feeble ETS exposures have been postulated only by presuming what is merely possible, even if extremely unlikely, as opposed to what is scientifically demonstrable and probable. Although at times politically tempting, such hypothetical presumptions are not science and should be resisted. If accepted, they are likely to foster irrational fears, not the enlightened prudence that responsible public health policy should cultivate.

TABLE 6

REPORTED INDEPENDENT RISK FACTORS FOR LUNG CANCER

Factor	Reference	Maximum RR reported	95% CI
Family history of lung cancer	Samet *et al.* (1986)	5.3	(2.2–12.8)
	Ooi *et al.* (1986)	2.4	
	Horwitz *et al.* (1988)	2.8	(1.0–7.7)
	Wu *et al.* (1988)	3.9	(2.0–7.6)
Family history of tuberculosis	Wu *et al.* 1988)	10.0	(1.1–90.1)
	Sakurai *et al.* (1989)	6.4	
	Gao *et al.* (1987)	1.7	(1.1–2.4)
	Hinds *et al.* (1982)	8.2	(1.3–54.4)
β-carotene/vitamin A deficiency	Byers *et al.* (1987)	0.3	(P = 0.06 trend)
	Pastorino *et al.* (1987)	0.2	
	Wu *et al.* (1985)	0.4	(0.2–0.9)
	Ziegler *et al.* (1986)	2.2	
Alcohol intake	Pollack *et al.* (1984)	2.19	(1.3–5.0)
Dietary cholesterol/fat	Goodman *et al.* (1988)	2.2	(1.3–3.8)
Dietary fat intake	Wynder *et al.* (1987)	4–6	
Pork meat intake	Mettlin (1989)	2.4	(1.4–4.2)
Vegetable diet	Jain *et al.* (1990)	0.6	(0.4–0.88)
	Le Marchand *et al.* (1989)	0.3	(P = 0.009 trend)
Fruit intake	Koo (1988)	0.4	(0.2–0.9)
Milk intake	Mettlin (1989): Mettlin *et al.* (1990)	2.1	(1.4–3.2)
Hormone therapy in women	Adami *et al.* (1989)	1.3	
Cooking methods	Gao *et al.* (1987)	1.4–2.6	(1.1–5.0)
	Geng *et al.* (1988)	5.6	(3.4–9.1)
	Sobue *et al.* (1990)	1.9	(1.1–3.3)
	Mumford *et al.* (1987)	2–3	
Radon	Edlin *et al.* (1984)	4.3	(1.7–10.6)
	Lees *et al.* (1987)	2.4	(0.8–7.1)
Occupation	Kvale *et al.* (1986)	2.6	
Motor exhaust exposure	Hayes *et al.* (1989)	1.5	(1.2–1.9)
Socioeconomic class	Brown *et al.* (1975)	2.6–3.8	
Ventilatory function	Lange *et al.* (1990)	2–4	
Cardiac anomalies	Tenkanen *et al.* (1987)	2.4	
Physical inactivity	Albanes *et al.* (1989)	1.6	(1.2–3.5)
	Severson *et al.* (1989)	1.4	(1.0–2.1)
Psychosocial traits	Kulessa *et al.* (1989)	2–3	
Urban/rural risk ratio	Shy (1984)	1.2–2.8	

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SECTION 3

ETS AND LUNG CANCER

Attempts to Quantify Exposure:

- **Questionnaires**
- **Chemical Markers**
- **Personal Monitors**
- **Monitoring in Real Life Situations**

SCOTH REVIEW - VOLUME 3

SECTION 3

ATTEMPTS TO QUANTIFY EXPOSURE : QUESTIONNAIRES, CHEMICAL MARKERS, PERSONAL MONITORS AND MONITORING IN REAL-LIFE SITUATIONS

A wide range of experimental studes have been conducted in an attempt to find reliable methods for quantifying exposure to ETS. Each such method has been reported to have limitations. Thus while the various methods described in the papers in this section may have useful practical applications, none of them has been generally acknowledged as providing a consistently accurate measure of actual ETS exposure.

The epidemiological studies of ETS and lung cancer have typically estimated exposure by the use of questionnaires. The reliability of this approach is highly questionable (Proctor, Defino, Pron). An alternative approach which was developed tried to assess ETS exposure by measuring ambient concentrations of chemical markers such as nicotine and carbon monoxide (CO). Researchers have however identified problems in the use of such "surrogates". The ratio of nicotine to other ETS constituents is, for example, highly variable (Nelson), while ambient CO has many sources other than ETS.[2]

Recent developments involving the use of personal monitors to assess exposure to tobacco-specific constituents may provide a more useful guide to ETS exposure. One such study (Phillips et al), while expressing reservations about the concept of "cigarette equivalents", estimated that non-smokers may be, on average, exposed to the equivalent of about 5 cigarettes per annum. While personal monitoring like any other method may have its own problems, the technique clearly comes closest to

[2] Nicotine and CO are not the only surrogates which have been proposed for ETS. A discussion of other possible markers, and their limitations can be found in Guerin et al, *The Chemistry of Environmental Tobacco Smoke* which is submitted under separate cover.

evaluating actual exposure, a key to assessing any real, as opposed to speculative, risk. Some of the studies which have used both questionnaires and objective measurement have confirmed a substantial degree of smoker/non-smoker misclassification.

Finally, this section includes a number of studies which have attempted to measure ETS concentrations in a variety of "real-life" situations. Whatever the limitations of the methodologies used, the studies show that overall concentrations of ETS in these differing situations are generally low.

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SECTION 3

ATTEMPTS TO QUANTIFY EXPOSURE: QUESTIONNAIRES, CHEMICAL MARKERS, PERSONAL MONITORS AND MONITORING IN REAL-LIFE SITUATIONS.

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* Not peer reviewed

SECTION 3

ETS AND LUNG CANCER

QUESTIONNAIRES

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A COMPARISON OF METHODS OF ASSESSING EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE IN NON-SMOKING BRITISH WOMEN

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Fifty-two non-smoking British women were recruited to wear personal monitors for nicotine and volatile organic compounds over a 24-h period in the autumn of 1989. The subjects also supplied samples of saliva for cotinine analysis, and answered questions regarding lifestyle and exposure to environmental tobacco smoke (ETS). The research indicates that exposure to ETS in free-living subjects is very low (mean nicotine exposure 2.3 $\mu g\ m^3$). Moreover, the greatest influence on exposure was living with a smoker, contact with smokers at work; leisure or travel having a minor impact. Salivary cotinine levels were found in subjects observed not to be exposed to ETS, hence somewhat questioning the validity of this measure for very low levels of exposure. Both of the objective measures of ETS exposure, nicotine and salivary cotinine levels, only correlated modestly with subjective assessments of exposure obtained by questionnaire.

INTRODUCTION

Environmental tobacco smoke (ETS) is the complex and dilute mixture of substances found in indoor air as a specific result of tobacco smoking. Through the 1980's, ETS has been the subject of much research, principally because of claims that exposure to ETS might be harmful to non-smokers (U.S. Surgeon General 1986).

An important consideration in any study of ETS, whether epidemiological, risk-assessment or laboratory-based research, is the determination of the populations' actual exposure to ETS (Proctor and Smith 1989). It is clearly not possible to absolutely measure a person's lifetime exposure to ETS and so estimates are based on either subjective or single point objective measures.

In epidemiology, the method of exposure assessment is either by questioning the spouse or a relative of the subject, or by reference to hospital records. This is likely to result in an indication as to whether the subject was exposed to ETS in the home, but is unlikely to produce an accurate assessment of actual exposure (Coultas et al. 1989).

In some studies of ETS and lung cancer, the number of cigarettes smoked by the spouse per day is used to segregate exposure levels in attempts to find dose-response relationships (Hirayama 1981; Trichopoulos et al. 1981; Garfinkel 1981; Chan and Fung 1982). Other studies (Correa et al. 1983; Miller 1984; Garfinkel et al. 1985; Kabat and Wynder 1984; Wu et al. 1985) also included questions about whether exposure had occurred at work (yes/no) and/or whether parents had smoked (yes/no). However, as Koo et al. (1987) suggest, these somewhat crude indices of exposure do not take into account that the degree of

contact of the non-smoker with these smokers could be very low, or even nil.

Potential levels of exposure can be determined by fixed site monitoring. For this, typically time-weighted average concentrations of ETS related substances (usually nicotine and the contribution of ETS to respirable suspended particulates) are acquired in a certain type of environment. ETS levels will be dependant upon many factors, such as the rate of smoking, size of environment and ventilation conditions. Hence, for such measurements to be applicable to general populations, efforts must be made to ensure that the environments sampled are representative and that substances collected to assess ETS are specific (and do not include contributions from other sources) (Proctor 1988; Proctor and Smith 1989).

Even combining fixed site monitoring with time-activity considerations can be misleading. For example, exposure to ETS in the home will depend upon, to some extent, both the time spent in the same room as the smoker and the proximity of each contact. Hence it is likely that personal monitoring, where ETS substances are continuously measured close the breathing zone of the subject, will give a more accurate determination of exposure.

A further step is to measure the metabolite of an ETS related substance (such as cotinine, one of the metabolites of nicotine) in either plasma, urine or saliva (Haley et al. 1989a; Henderson et al. 1989). However, even these measures require careful interpretation, especially if single rather than cumulative samples are acquired, as there seems to be considerable inter-subject variability in nicotine metabolism (Wall et al. 1988), and because of the possible impact of the time of day of sample collection (Jarvis et al. 1987).

This study reports the findings of a comparison of three exposure assessment methods in a group of 52 free-living British non-smoking women. Each of the subjects was questioned on both, perception of exposure to ETS and on observation of cigarette smoking around them, whilst also wearing a personal monitor for airborne nicotine and supplying saliva for cotinine analysis. The personal monitor also allowed quantitation of exposure to volatile organic compounds (VOCs). Hence, for each subject, information was obtained on actual exposure, associated metabolite levels and perceived exposure to ETS. By comparing the data acquired from each of the approaches, the study allows an insight into the limitations of using any one of the exposure assessment methods in isolation.

PROCEDURAL CONSIDERATIONS

Sample selection

Subjects were selected independently by a market research agency (John Mumford Associates, London). The preset criteria was to acquire a sample balanced by living with a smoker or not and by working or not. An even distribution by age and social classification was also attempted for each sub group. All subjects were non-smoking women (at time of interview), living in either the Birmingham or Fordingbridge areas of England. All samples were acquired in November and December of 1989.

Some 70 subjects were studied, but 18 were rejected from the analysis because of either personal monitor failure or because insufficient saliva was collected. The remaining 52 were classified as in Table 1.

Table 1. Classification of sample.

	Total No. of Subjects	Age				Social Classification			
		18-24	25-34	35-44	45-54	AB	C1	C2	DE
Smoking home/working	12	3	2	2	5	2	2	7	1
Smoking home/not working	11	3	4	2	2	3	3	4	1
Non-smoking home/working	12	1	5	1	5	0	6	3	3
Non-smoking home/not working	17	3	6	7	1	2	5	6	4

The subject acquisition was semi-random. For the selection of each subject, an area of social classification was identified prior to interviewers calling door-to-door, asking for non-smokers willing to partake in the study. Once a subject was identified, a date was set for the study day.

On the day of study, the interviewer arrived at a time convenient to the subject, instructed on the use of the personal monitor, placed, and switched the monitor on. At this time, a first saliva sample was taken. The subject then wore the monitor for 24 h (placing it at bedside when sleeping). Subjects also carried activity diaries where they noted times of leaving the house, traveling, working and when they were exposed to tobacco smoke. They also recorded the observed number of cigarettes smoked around them, separated by environmental type. After this 24-h period, the interviewer returned, switched off the monitor and took a second saliva sample. The subject was then questioned on the type of cooking, heating, and various lifestyle events relevant both to the monitoring period and in general.

Personal monitoring

The personal monitoring apparatus consisted of a sampling tube connected to a fixed volume diaphragm pump. The collection tube (ATD50 tube, Perkin-Elmer Ltd., U.K.) was a 9-cm-long stainless steel tube filled with about 0.2 g of Tenax TA adsorbant (30-60 mesh) (Chrompack, U.K.). Each tube had been thoroughly conditioned prior to use to ensure lowest possible background contamination. The tube was held close to the breathing zone by means of an SKC holder attached to a short chain worn around the neck. Subjects were instructed to ensure that the tube continued to protrude when overcoats were worn. The tube was connected by flexible tygon tubing to a sampling pump (SKC Model 224-4) held around the waist on a belt. These pumps have visible counters that indicate the volume sampled. The volume per count for each pump was calibrated (Gilibrator Automated Calibrator, Model D800286)) before and after sampling. Sampling pumps were tested prior to the study for retention of calibrated flow over 24-h periods. Similarly, breakthrough characteristics of the tubes under these conditions were tested. Typically, a total of 25 L was sampled using a constant flow rate over the 24-h period.

After sampling, the tubes were capped and stored in a freezer until transfer (cold) to our laboratory at the end of the study. Analysis of each tube involved procedures previously described in detail and validated for nicotine and VOC analysis (Thompson et al. 1989; Proctor et al. 1989). In brief, each tube was desorbed for 15 min at 240°C with helium gas flushing the released chemicals onto a cold trap containing a small amount of Tenax and maintained at -30°C. After this primary desorption, the trap was electronically heated to 240°C, effectively injecting the compounds on to the head of a 30 m, 0.32 μm I.D., DB5 capillary gas chromatographic column. This column separates the compounds of interest, which were subsequently detected, identified, and quantified using a bench-top mass spectrometer (Finegan Ion Trap Detector). Ion-specific detection was used for quantification (i.e., nicotine was quantified on the m/z 84 base peak of the mass spectrum). Calibration was achieved by injecting known quantities of nicotine and VOCs at five concentration levels on to clean Tenax tubes. Hence, standards followed the same analysis route as did the samples.

Salivary cotinine collection and analysis

Dental rolls (Claudius Ash and Sons, U.K.), were used to collect saliva. Subjects were asked to place a clean dental roll in their mouth, between their upper gums and cheek, for 30 min at the beginning (T_1) and again at the end (T_2) of the monitoring period. After this time, the roll was transferred into a coded vial subsequently placed in a cool box until the interviewer returned home, when it was then stored in a freezer.

At the end of the study, all saliva samples were transferred cold to New Cross Hospital, London, where they were analysed blind of subject information by an up-date of the method developed by Feyerabend et al. (1986). This involved basified extraction of the saliva collected by squeezing the dental roll, followed by analysis of the extract by capillary gas chromatography with nitrogen-phosphorus detection.

RESULTS AND DISCUSSION

The data are summarised in Table 2. Means, medians, and ranges are given for salivary cotinine values before (T_1) and after (T_2) the monitoring period, and time-weighted exposure concentrations for nicotine and some volatile organic compounds. Furthermore, both the subjects' observation of the number of cigarettes (including other tobacco products) smoked in proximity either at home or at work totalled to include travel and leisure and their subjective assessment of the extent of exposure to ETS, automobile exhausts, and general air pollution is given. The data set is summarised both for all subjects and segregated by smoking/non-smoking household and by subject working/not working.

Table 2. Summary of data for all subjects and segregated by smoking/nonsmoking household and by working/not working.

		Smoking Household/Working	Smoking Household/ Not Working	Non-smoking Household/ Working	Non-smoking Household/ Not Working	All Subjects
[1]Total number of cigarettes	Mean	7.4	6.6	5.4	0.4	4.5
	Median	5.5	5.0	2.0	0	2
	Range	1-25	1-30	0-50	0-2	0-50
[2]Number of cigarettes at home	Mean	4.7	6.3	0	0.2	2.5
	Median	2	4	0	0	0
	Range	0-25	1-30	0-0	0-2	0-30
[3]Number of cigarettes at work	Mean	1.5	0	5.4	0	1.6
	Median	0	0	2	0	0
	Range	0-10	0-0	0-50	0-0	0-50
[4]Salivary cotinine T_1 (ng ml^{-1})	Mean	2.6	2.5	1.3	1.1	1.8
	Median	1.2	2.2	1.15	0.9	1.2
	Range	0.3-15.1	0.5-8.1	0.3-3.5	0.3-2.9	0.3-15.1
[5]Salivary cotinine T_2 (ng ml^{-1})	Mean	1.7	2.2	1.3	1.1	1.5
	Median	1.3	1.5	1.0	0.8	1.1
	Range	0.1-5.3	0.2-9.0	0-4.4	0.2-3.7	0-9.0
[6]Nicotine ($\mu g\ m^{-3}$)	Mean	1.6	7.4	0.8	0.5	2.3
	Median	0	2.3	0.4	0	0
	Range	0-9.6	0-45.4	0-2.6	0-7.2	0-45.4
Benzene ($\mu g\ m^{-3}$)	Mean	15.7	21.6	60.7	13.2	26.5
	Median	13.3	13.4	15.5	10.4	12.8
	Range	3.2-48.7	5.2-103	0.7-510	0.2-32.1	0.2-510
Toluene ($\mu g\ m^{-3}$)	Mean	272	112	262	144	194
	Median	85	120	94	78	89
	Range	39-2191	22-208	0.4-1589	0.2-1264	0.2-1264

1 Total number of cigarettes is the subjects' assessment of the number of cigarettes smoked in proximity over the sampling period.
2 Number of cigarettes at home is the subjects assessment of the number of cigarettes smoked whilst at home during the sampling period.
3 Number of cigarettes at work is the subjects' assessment of the number of cigarettes smoked in proximity whilst at work during the sampling period.
4 Salivary cotinine T_1 relates to the sample taken at the onset of the sampling period.
5 Salivary cotinine T_2 relates to the sample taken directly after the 24 hour sampling period.
6 All VOC concentrations are time weighted averages over the 24 hour period and are given in $\mu g\ m^{-3}$. 0 is equivalent to a detection limit of 0.1 $\mu g\ m^{-3}$.

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Table 2. Continued.

		Smoking Household/Working	Smoking Household/ Not Working	Non-smoking Household/ Working	Non-smoking Household/ Not Working	All Subjects
Ethylbenzene ($\mu g\ m^{-3}$)	Mean	10.2	10.1	12.5	11.1	11.0
	Median	9.0	11.3	12.3	9.8	9.7
	Range	5.9-19.5	4.0-14.6	0.1-30.4	0.3-52.1	0.1-52.1
o-Xylene ($\mu g\ m^{-3}$)	Mean	12.1	10.2	13.7	9.5	11.2
	Median	9.2	8.9	12.9	10.5	9.4
	Range	1.1-49.6	3.8-16.4	1.0-40.9	1.0-17.0	1.0-40.9
m/p-Xylene ($\mu g\ m^{-3}$)	Mean	37.2	30.6	47.9	34.0	37.2
	Median	22.2	21.8	44.3	17.3	24.4
	Range	16.1-159	4.7-102	3.0-127	3.4-166	3.0-166
Styrene ($\mu g\ m^{-3}$)	Mean	3.2	3.0	9.5	2.9	4.5
	Median	2.0	2.3	2.5	2.1	2.2
	Range	1.4-12.6	1.0-9.7	0.3-49.8	0.8-12.4	0.3-49.8
Chlorobenzene ($\mu g\ m^{-3}$)	Mean	0.2	0.2	0.2	0.5	0.3
	Median	0.1	0.1	0.2	0.1	0.1
	Range	0.1-0.4	0.1-0.5	0.1-0.7	0.1-5.2	0.1-5.2
1,4-dichlorobenzene ($\mu g\ m^{-3}$)	Mean	8.8	12.2	12.8	1.5	8.1
	Median	0.7	0.7	2.5	0.6	0.7
	Range	0.2-89.3	0.2-81.7	0.1-114	0.3-6.5	0.1-114
n-Octane ($\mu g\ m^{-3}$)	Mean	11.0	7.8	15.5	12.4	11.8
	Median	9.1	5.9	7.4	7.0	7.0
	Range	3.5-32.7	1.7-22.2	0.1-55.3	2.4-77.4	0.1-77.4
N-Decane ($\mu g\ m^{-3}$)	Mean	16.2	21.8	67.2	27.6	32.8
	Median	13.8	14.3	14.8	12.2	13.9
	Range	1.9-66.6	2.5-62.2	3.5-484	1.8-80.3	1.8-484
n-Undecane ($\mu g\ m^{-3}$)	Mean	8.4	9.9	29.7	10.5	14.3
	Median	6.9	10.1	15.5	4.3	8.4
	Range	1.3-34.5	2.2-29.9	2.5-163	2.3-35.4	1.3-163
n-Dodecane ($\mu g\ m^{-3}$)	Mean	4.7	5.9	7.2	5.8	5.9
	Median	3.1	2.9	5.2	3.1	3.6
	Range	0.7-15.5	0.8-21.1	0.6-28.3	0.7-30.9	0.6-30.9
Limonene ($\mu g\ m^{-3}$)	Mean	18.4	16.9	13.4	15.9	16.1
	Median	14.3	10.5	11.6	12.3	12.5
	Range	1.6-47.3	0.3-45.0	0.4-53.1	0.4-72.0	0.3-72.0

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Table 2. Continued.

		Smoking Household/Working	Smoking Household/ Not Working	Non-smoking Household/ Working	Non-smoking Household/ Not Working	All Subjects
α-pinene ($\mu g\ m^{-3}$)	Mean	8.6	21.4	7.6	11.8	12.1
	Median	7.8	9.0	6.6	7.6	7.6
	Range	3.2-18.4	2.4-116	1.4-18.5	2.1-76.2	0.6-116
Chloroform ($\mu g\ m^{-3}$)	Mean	0.6	1.0	1.2	1.4	1.1
	Median	0.5	0.7	0.7	0.7	0.7
	Range	0.1-2.5	0.2-2.1	0.1-4.5	0.1-11.9	0.1-11.9
1,1,1-trichloro ethane ($\mu g\ m^{-3}$)	Mean	476	8.6	20.6	6.9	119
	Median	15.5	4.9	5.6	6.8	6.2
	Range	2.8-2507	2.0-45.5	1.3-172	0.1-15.1	0.1-2507
1,2-dichlorethane ($\mu g\ m^{-3}$)	Mean	0.2	0.4	3.7	0.4	1.1
	Median	0.3	0.4	0.4	0.4	0.4
	Range	0.1-0.5	0.2-0.6	0.1-40	0.1-1.4	0.1-40
Trichlorethene ($\mu g\ m^{-3}$)	Mean	9.1	8.3	10.2	6.9	8.5
	Median	8.7	7.2	7.8	5.4	6.5
	Range	1.0-31.2	2.0-20.1	0.4-4.5	0.1-11.9	0.1-31.2
Tetrachloroethene ($\mu g\ m^{-3}$)	Mean	3.6	3.3	14.0	1.9	5.4
	Median	1.6	2.0	2.4	1.4	1.9
	Range	0.9-12.4	0.8-13.9	0.2-76.8	0.1-6.0	0.1-76.8
Pyridine ($\mu g\ m^{-3}$)	Mean	0.8	1.6	0.8	0.5	0.9
	Median	0.5	0.8	0.4	0.3	0.5
	Range	0.2-2.4	0.4-8.9	0.2-3.8	0.2-2.2	0.2-8.9
[7]Assess ETS (1-5)	Mean	3.2	2.8	2.3	1.3	2.3
	Median	3	3	2	1	2
	Range	1-5	1-5	1-5	1-3	1-5
[8]Assess Car/bus (1-5)	Mean	3.1	2.4	2.6	2.5	2.6
	Median	3.5	2	2	2	2
	Range	1-5	1-5	1-5	1-5	1-5
[9]Assess all pollutibn (1-5)	Mean	2.7	2.3	2.6	2.2	2.3
	Median	2.5	1.0	2.0	2.0	2.0
	Range	2-4	1-4	1-5	1-5	1-5

7 Assess ETS is the subjects' assessment of exposure during the monitoring period to tobacco smoke in the air, scaled from 1 (none) to 5 (extreme).

8 Assess car/bus is the subjects' assessment of exposure to car and bus exhausts, scaled from 1 (none) to 5 (extreme)

9 Assess all pollution is the subjects' assessment of exposure to air pollution (other than car/bus exhausts), scaled from 1 (none) to 5 (extreme).

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Representativeness of sample

It is clearly important to assess whether wearing the monitor influenced the behaviour of the subject and, hence, whether the results are typical. All subjects were questioned as to whether their lifestyle during the monitoring period was typical and all but one, who did not wish to be seen wearing the monitor, said that it was.

With regard to exposure to ETS, one check is to see whether salivary cotinine values taken at T_1 and T_2 were similar (i.e., exposure was similar both on the day of study and the previous day). The median difference between T_1 and T_2 was found to be zero (mean -0.2 ng mL^{-1}, lower quartile -0.4 ng mL^{-1}, upper quartile 0.4 ng mL^{-1}, range -9.8 to 2.7). As shown in Table 3, the correlation between T_2 and T_1 was 0.73 ($r^2 = 0.53$) for all subjects and 0.75 ($r^2 = 0.56$) for smokers' homes. The largest disparity was found in a subject exposed to an observed value of 20 cigarettes at home, whose salivary cotinine dropped from 15.1 ng mL^{-1} (T_1) to 5.3 mg mL^{-1} (T_2). However, on the whole, it seems that the sampling system did not significantly impact their lifestyle.

Exposure to airborne nicotine

The highest mean (7.4 $\mu g\ m^{-3}$) and median (2.3 $\mu g\ m^{-3}$) time-weighted exposures to nicotine were found in non-working women living in homes with smokers. The maximum exposure (45.4 $\mu g\ m^{-3}$) was also found in this category. Levels were much lower in smoker homes where the subject was working (mean 1.6 $\mu g\ m^{-3}$, median 0, range O to 9.6 $\mu g\ m^{-3}$). No nicotine was detected in a significant number of these subjects, the limit of detection being 0.1 $\mu g\ m^{-3}$. However, as this is time-weighted, it is possible that exposure to higher concentrations than 0.1 $\mu g\ m^{-3}$ during part of the day could still result in the time weighted average being below the limit of detection.

The mean nicotine concentration for working subjects living in non-smoking homes, was 0.8 $\mu g\ m^{-3}$ (median 0.4, range 0-2.6 $\mu g\ m^{-3}$). Subjects neither working nor exposed to ETS at home had the lowest exposure (mean 0.5, median O and range 0-7.2 $\mu g\ m^{-3}$). This exposure presumably only results from either travel or leisure.

Across all subjects, the mean exposure to nicotine was 2.5 $\mu g\ m^{-3}$, with a median below 0.1 $\mu g\ m^{-3}$. The data seems to correspond to that found by McCarthy et al. (1987) where children from smoking families were found to be exposed to means of 2.5 $\mu g\ m^{-3}$ whilst those from non-smoking families were exposed to 0.3 $\mu g\ m^{-3}$ nicotine.

Our results suggest, as has been indicated by previous studies (Haley et al. 1989b), that the most influential source of exposure to ETS is in the domestic environment and that contributions at work, leisure or during travel are much smaller. Moreover, mean exposures are low. Some authors (Oldaker and Conrad 1987; Carson and Erikson 1988; Proctor et al. 1989) have attempted to put the levels of ETS constituents in perspective through cigarette equivalent calculations. This exercise takes the median exposure to airborne nicotine and typical breathing rates to give a daily exposure. This is then compared to the delivery of nicotine that would be obtained from smoking one cigarette. Such calculations are strictly an estimate of exposure, not dose, are only relevant to nicotine and not ETS as a whole, and take no account of the differences between breathing air and inhaling smoke. Taking a typical female breathing rate of 0.62 $m^{-3}\ h^{-1}$ (Arundel et al. 1987) and a mainstream nicotine delivery of 1.3 mg cig^{-1} for a typical U.K. cigarette, then the median daily exposure over all subjects would be less than the nicotine equivalent of 0.001 cigarette. That means that the non-smoking subject on the average would only be exposed to the equivalent nicotine of smoking a single cigarette after a period in excess of 2 y.

Salivary cotinine levels

The highest mean (2.2 ng mL^{-1}) and median (1.5 ng mL^{-1}) T_2 salivary cotinine levels were found in non-smoking subjects living in smoker households. Working subjects living with smokers had a T_2 mean of 1.7 ng mL^{-1} (median 1.3 ng mL^{-1}, whilst those living in non-smoker households had a T_2 mean of 1.3 ng mL^{-1} (median 1.0 ng mL^{-1}). Subjects that neither worked nor lived with a smoker had the lowest T_2 mean of 1.1 ng mL^{-1} (median 0.8 ng mL^{-1}) Hence, these trends follow the results found in the personal monitoring data for nicotine.

However, salivary cotinine levels were observed in all but one subject. In 30 of the 52 subjects, there was no detectable exposure to nicotine. This, at first inspection, seems to indicate that the salivary cotinine measure is more sensitive to nicotine exposure than personal monitoring. It should be expected though that detection of salivary cotinine would be associated with an observation of cigarettes being smoked in proximity to the subject. But in 15 cases, the subjects observed no exposure to ETS and yet salivary cotinine was detected. Moreover, the levels in these cases (mean 1.0 ng mL^{-1}; median 0.85 ng mL^{-1}; range 0.5-3.7 ng mL^{-1}), overlap with cases where ETS exposure was observed. This might be

Table 3. Correlations and regression analysis.

		Corr.	r^2	F	P
Salivary cotinine T_1 and salivary cotinine T_2	(All subjects)	0.73	0.53	55.3	0
Salivary cotinine T_1 and salivary cotinine T_2	(Smokers' households)	0.75	0.56	26.9	0
Salivary cotinine T_1 and salivary cotinine T_2	(Non-smokers' households)	0.59	0.34	14.4	0.001
Salivary cotinine T_1 and nicotine	(All subjects)	0.49	0.24	15.5	0
Salivary cotinine T_2 and nicotine	(All subjects)	0.71	0.51	52	0
Salivary cotinine T_1 and nicotine	(Smokers' households)	0.45	0.2	5.3	0.032
Salivary cotinine T_1 and nicotine	(Non-smokers' households)	0.32	0.1	3.1	0.09
Salivary cotinine T_2 and nicotine	(Smokers' households)	0.79	0.63	35	0
Salivary cotinine T_2 and nicotine	(Non-smokers' households)	0.21	0.04	1.3	0.27
Observed total number of cigarettes and nicotine	(All subjects)	0.5	0.25	16.4	0
Observed total number of cigarettes and nicotine	(Smokers' households)	0.74	0.55	25.3	0
Observed total number of cigarettes and nicotine	(Non-smokers' households)	0.29	0.09	2.5	0.125
Observed total number of cigarettes and salivary cotinine T_2	(All subjects)	0.63	0.39	32.5	0
Observed total number of cigarettes and salivary cotinine T_2	(Smokers' households)	0.70	0.49	20.4	0
Observed total number of cigarettes and salivary cotinine T_2	(Non-smokers' households)	0.62	0.38	16.7	0
Observed total number of cigarettes smoked at home and nicotine	(Smokers' households)	0.80	0.64	37.2	0
Observed total number of cigarettes smoked at home and salivary cotinine T_2	(Smokers' households)	0.69	0.48	19.3	0
Assessed exposure to ETS and nicotine (all subjects)	(All subjects)	0.42	0.17	10.7	0.002
Assessed exposure to ETS and salivary cotinine T_2	(All subjects)	0.50	0.25	16.6	0
Assessed cigarettes per day and nicotine	(Smokers' households)	0.57	0.32	9.8	0.005
Assessed cigarettes per day and salivary cotinine T_2	(Smokers' households)	0.53	0.28	8.3	0.009
Benzene and nicotine	(All subjects)	0.05	0.002	0.12	0.726
Benzene and nicotine	(Smokers' households)	0.304	0.09	2.13	0.159
Benzene and salivary cotinine T_2	(All subjects)	0.137	0.002	0.95	0.334
Benzene and salivary cotinine T_2	(Smokers' households)	-0.009	0.0	0	0.969

Corr = Pearson correlation (r)
r^2 = Square of the correlation
F = F ratio, the mean square factor/mean square error
P = Probability of F occurring by chance

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explained by ETS exposure during previous days, but, if this were to be the case, then it would be expected that the level at T_2 would be less than that at T_1. In fact, for 10 of these 15 cases, T_2 was higher than T_1. This raises doubts about the validity of the salivary cotinine information at low levels and suggests that studies that have suggested large proportions of the population being exposed to ETS may be misleading (Repace and Lowry 1985; Wells 1988).

Comparing personal exposure to nicotine with salivary cotinine

Table 3 shows that for the full data set, the correlation between salivary cotinine at T_2 and nicotine was 0.71 ($r^2 = 0.51$, $F = 52$). When restricted to smoker households, this increases to a correlation of 0.79 ($r^2 = 0.63$, $F = 35$). In non-smoker households, the correlation of 0.21 ($r^2 = 0.04$, $F = 1.3$) is not significant. However, here many of the nicotine data points were below the detection limit.

Salivary cotinine at T_1 correlates with nicotine at 0.49 ($r^2 = 0.24$, $F = 15.5$) for all subjects, but this reduces to 0.45 ($r^2 = 0.2$, $F = 5.3$) in smoker households. Hence, it seems possible to correlate salivary cotinine levels taken after monitoring with personal nicotine exposures, but only in the higher exposure group of subjects living with smokers.

Some authors have used salivary cotinine data to interpret exposures in terms of cigarette equivalents (Jarvis et al. 1987). Using typical salivary cotinine levels in a 20 cigarettes/d smoker of 300 ng ml, it was suggested that levels found in non-smoking children were as high as the equivalent of one half of a cigarette/d. Carrying out the same calculation on our data, would suggest that the highest exposed group (living with smokers, not working), would be receiving the mean equivalent nicotine dose of 0.15 cigarettes/d.

However, if one again takes the mean exposure to nicotine over the 24-h period, using a female respiration rate of 0.62 m^{-3} h^{-1} and a typical mainstream nicotine delivery of 1.3 mg cig^{-1}, then this exposure is the nicotine equivalent of smoking 0.08 cigarettes/d. This disparity is further illustrated in the non-working subjects in non-smoking homes where calculations based on salivary cotinine would suggest cigarette equivalents of 0.08 cigarettes/d, whilst those based on nicotine exposure suggest 0.006 cigarettes/d. The difference, as Haley et al. (1989b) suggest, is likely due to the salivary cotinine calculation not taking into account the different metabolic clearance rates of cotinine in smokers and non-smokers.

Correlating objective and subjective evaluations of ETS exposure

It might be expected that the observed number of cigarettes smoked close to the subject would positively correlate with both measures of personal nicotine exposure and salivary cotinine levels at T_2. When taking all subjects, the correlation between total cigarettes and nicotine is 0.5 ($r^2 = 0.25$, $F = 16.4$), and with salivary cotinine T_2 is 0.63 ($r^2 = 0.39$, $F = 32.5$); hence, there is a modest correlation. If the data set is restricted to the numbers of cigarettes smoked at home in smoking households, then the correlation with nicotine is much stronger at 0.8 ($r^2 = 0.64$, $F = 37.2$). With salivary cotinine T_2, this correlation is not so good, at 0.69 ($r^2 = 0.48$, $F = 19.3$).

Two further assessments of ETS exposure were acquired by questionnaire. The first was to ask, prior to monitoring, how many cigarettes were typically smoked per day by the smoker, a question often used in epidemiological studies. This assessment correlated relatively poorly with nicotine exposure at 0.57 ($r^2 = 0.32$, $F = 16.6$). With salivary cotinine T_2 the correlation was 0.51 ($r^2 = 0.25$, $F = 17.5$). This assessment of only a modest correlation between objective and subjective measures of ETS exposure is in agreement with recent work presented by Coultas et al. (1989).

All subjects were also asked to assess their exposure to ETS scaled from 1 (none) to 5 (extreme) during the sampling period. This again, did not correlate well with either nicotine or salivary cotinine T_2 levels.

Exposure to volatile organic compounds

The 24-h time-weighted average exposures to a range of VOCs are given in Table 2. The concentrations found are broadly similar to the winter/indoor data published in the U.S. Environmental Protection Agency's (EPA) Total Exposure Assessment Methodology (TEAM) studies (Pellizari et al. 1986; Wallace 1987). For example, the TEAM study found median personal exposure levels of benzene during daytime at 11 μg m^{-3} (Wallace 1987), whilst the median benzene level for all of our subjects was 13 μg m^{-3}. Our study also used a similar analytical methodology to that utilised in TEAM studies.

Overall, neither working nor smoking had a dramatic effect on personal exposures to VOCs. However, there are some trends. Taking differences between means for smoking/non-smoking and working/non-working to be equivalent if the difference was less than 10% of the mean of all for a particular

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compound, then there are increased levels of exposure for subjects working over subjects not working for 13 of the 21 VOCs analysed. This includes benzene (mean working of 38 $\mu g\ m^{-3}$, not working 16 $\mu g\ m^{-3}$), toluene (mean working of 267 $\mu g\ m^{-3}$, not working 131 $\mu g\ m^{-3}$), styrene (mean working of 6 $\mu g\ m^{-3}$, not working 3 $\mu g\ m^{-3}$) and 1,1,1-trichloroethane (mean working 248 $\mu g\ m^{-3}$, not working 7 $\mu g\ m^{-3}$). For five of the VOCs, means were higher for subjects not working, including nicotine (not working 3.2 $\mu g\ m^{-3}$, working 1.2 $\mu g\ m^{-3}$) and α-pinene (not working 16 $\mu g\ m^{-3}$, working 8 $\mu g\ m^{-3}$). For three further VOCs, exposures were equivalent. However, none of the differences found were statistically significant.

Comparing mean VOC data in a similar manner for subjects living in smoking households against non-smoking households, 6 VOCs were higher for the smoker household category. These included nicotine (smoking 4.3 $\mu g\ m^{-3}$, non-smoking 0.7 $\mu g\ m^{-3}$), limonene (smoking 18 $\mu g\ m^{-3}$, non-smoking 15 $\mu g\ m^{-3}$) and 1,1,1 trichloroethane (smoking 252 $\mu g\ m^{-3}$, non-smoking 13 $\mu g\ m^{-3}$). Subjects not living with smokers were exposed to higher means of 12 VOCs, including benzene (non-smoking 33 $\mu g\ m^{-3}$, smoking 19 $\mu g\ m^{-3}$), m/p xylene (non-smoking 40 $\mu g\ m^{-3}$, smoking 34 mg m^{-3}) and n-decane (non-smoking 44 $\mu g\ m^{-3}$, smoking 19 $\mu g\ m^{-3}$).

Means for each of the VOCs were separated by social classification [AB (high), C1, C2, DE (low)] of the subject. In general, exposures were evenly distributed by social class, though there was a trend of benzene and toluene exposures increasing with lower social class, and 1,1,1-trichloroethane and α-pinene increasing with higher social class.

Wallace (1989) has suggested that exposure to environmental tobacco smoke corresponds to significantly increased personal exposures to benzene, xylenes, ethylbenzene, and styrene. Several previous studies have suggested that this is unlikely (Bayer and Black 1987; Proctor et al. 1989). If it were to be the case, then it might be expected that there would be a strong correlation between personal exposures to nicotine and to these aromatic VOCs. As shown in Table 3, there is no significant correlation between either benzene and nicotine or between benzene and salivary cotinine (T_2) levels. The same holds true for xylenes, ethylbenzene, and styrene.

The highest observed exposure to benzene (510 $\mu g\ m^{-3}$) was some 40 times greater than the median exposure for all subjects. This was found in a subject that worked as a cashier in a garage forecourt. Benzene is a significant component of petrol fumes. This subject was also found to be exposed to nearly 18 times (1588 $\mu g\ m^{-3}$) the median exposure to toluene and higher than average exposure to ethylbenzene and m/p-xylene. Another subject, working as an accountant in a bedding material retail outlet, was exposed to the highest toluene level (2190 $\mu g\ m^{-3}$), the highest o-xylene level (50 $\mu g\ m^{-3}$), and much higher than average levels of 1,4-dichlorobenzene, n-decane, n-undecane, and styrene (39 $\mu g\ m^{-3}$).

One subject used an open wood fire for heating in the home. This seems to correspond to much higher than average exposure to octane (55 $\mu g\ m^{-3}$), decane (484 $\mu g\ m^{-3}$), and undecane (163 $\mu g\ m^{-3}$), though aromatic VOCs were not significantly higher than average. Neither the use of gas heating or whether or not the house was double-glazed had any significant effect on personal exposures to VOCs. However, gas cooking was associated with increased mean exposures to benzene, ethylbenzene, o-xylene, styrene, toluene, and 1,4-dichlorobenzene. All of the high levels of exposure to 1,1,1-trichloroethane occurred in subjects that both worked and were exposed to ETS at home. This solvent usually results from contact with industrial cleaning or degreasing operations (Droz et al. 1988), and one possible source is dry-cleaned clothes. However, subjects were questioned as to whether they visited a dry cleaner on the study day or whether the clothes they wore had been recently dry cleaned, but the response to both these questions was negative for all subjects. It is unclear what was the source or whether there were several sources, but it is unlikely that this exposure is related to ETS as there was only a modest correlation with nicotine or salivary cotinine (T_2). The subject experiencing the highest exposure to 1,1,1-trichloroethane worked as a receptionist, and a possible source is typing correction fluid.

CONCLUSIONS

Although this study has been based on a relatively small subject set, some clear trends are apparent. It seems that the main factor in exposure to environmental tobacco smoke is living with a smoker. Exposure at work, leisure, or travel seems to be minor. Overall, exposure to airborne nicotine was found to be low (mean 2.3 $\mu g\ m^{-3}$, median <0.1 $\mu g\ m^{-3}$).

Salivary cotinine levels tend to increase with exposure to airborne nicotine, but many subjects not exposed to ETS were found to have detectable salivary cotinine levels at values that overlapped with subjects that were exposed. This warrants further investigation. Also, cigarette equivalent calcula-

tions based on salivary cotinine levels did not correspond with similar calculations based on exposure to ETS.

The study evaluated various objective and subjective methods for assessing populations' exposure to ETS. It was found that questions regarding spouse smoking habits, as commonly used in epidemiological studies, did not correspond well with either levels of exposure to nicotine or salivary cotinine measurements, although trends did exist.

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Questionnaire assessments of recent exposure to environmental tobacco smoke in relation to salivary cotinine

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Questionnaire assessments of recent exposure to environmental tobacco smoke in relation to salivary cotinine. R.J. Delfino, P. Ernst, M.S. Jaakkola, S. Solomon, M.R. Becklake. ©ERS Journals Ltd 1993.

ABSTRACT: The increasing evidence of the ill-health effects of environmental tobacco smoke (ETS) has prompted the search for accurate measures of exposure to ETS. The present study examined whether it was possible to enhance the ability of questionnaire-derived assessments of ETS exposure, to predict salivary cotinine.

Salivary samples were obtained from 258 nonsmoking bank employees, who simultaneously answered questions detailing their exposure to second-hand smoke within the last three days. Exposure models were created, to take into account the number of smokers nearby, length of time in their presence, half-life of cotinine in bodily fluids, level of aversion to cigarette smoke and time of year.

All models, including the consideration of intensity and duration of exposure combined, explained an equal amount of variance of log cotinine levels (approximately 16%).

The weak relationship between questionnaire estimates of ETS exposure and cotinine, found in the present study, suggests that further investigation is needed to improve the assessment of recent ETS exposure.
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The accurate assessment of involuntary exposure to environmental tobacco smoke (ETS) has become important, in the light of growing evidence of its deleterious health effects [1, 2]. Questionnaire information has commonly been used to assess both acute and chronic exposure to ETS, and has been compared to objective measures of exposure, which include biological markers such as salivary cotinine, and air monitoring of ambient levels of nicotine and respirable particulates [3–14].

Previous studies have separately examined duration (hours exposed) and intensity (number of smokers) over varying periods of exposure (1–4 days) as covariables in relation to cotinine levels [10, 11, 14]. In a 10-country collaborative study of the determinants of cotinine levels [15], a comparison was made of questionnaire estimates of duration, intensity and cumulative exposure (cigarettes×time corrected for room volume), at home and at work, by women over the previous 4 days. The estimate of duration better predicted workplace exposure, whereas intensity better reflected home exposure [15]. When considering the cumulative index, each cigarette smoked by the husband in the woman's presence was equivalent to approximately two cigarettes smoked in her workplace. In none of the previous studies was an adjustment made for the half-life of cotinine, estimated to be between 20–40 h, and dependent upon variable metabolic rates between individuals.

The objective of the present study was to determine whether the ability of various questionnaire-derived estimates of ETS exposure to predict salivary cotinine, in both men and women, could be enhanced by considering detailed exposure information from the previous 3 days, and estimating a cumulative index which takes into account the half-life of cotinine.

Patients and materials

The subjects studied were part of a follow-up investigation of the effect of cigarette smoking on ventilatory lung function in young adults [16]. The study population consisted of 251 nonsmokers (no smoking for at least 5 months), who had given a salivary sample suitable for cotinine analysis and completed a questionnaire, out of 391 employees (140 current smokers) from two banks in Montreal and Toronto. Excluded were three subjects who claimed to be nonsmokers but whose cotinine levels were greater than 20 $ng \cdot ml^{-1}$; these subjects were considered likely to be "deceivers" and were dropped from the analysis, consistent with practice in previous studies [4, 9]. This left 248 nonsmoking subjects for analysis.

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Methods

Questionnaire data

The questionnaire was self-administered at the time salivary cotinine samples were collected, from April 1988 until October 1988. The questionnaire required approximately 15 min to complete, and included questions regarding personal smoking history, exposure to ETS over the period 1981–1988, and of direct concern to the present analysis, questions detailing the previous 3 days of ETS exposure.

For each of the prior 3 days (today, yesterday and the day before yesterday) and each potential place of exposure (work, home, vehicle, social setting and other) subjects were questioned on: 1) type of tobacco smoke exposure (cigarette, pipe and cigar smoke); 2) number of smokers within a 10 ft radius of the subject (intensity), set at a maximum value of five; and 3) duration of exposure in number of hours. Although the type of tobacco smoke was ascertained, they were treated equivalently, due to the rarity of pipe/cigar exposure in this population. Source identity (spouse, friend, *etc.*) for exposure was not ascertained.

Other questions included: 1) the time of day the saliva sample was obtained (morning or afternoon); 2) whether the subject was bothered by ETS (not at all, a little, moderately, or a lot); and 3) the number of hours spent outdoors today, yesterday and the day before yesterday. One subject had a missing value for the aversion variable, which was replaced using the value of three of five subjects with the same age and gender. Laboratory coding and computer entry of questionnaire data was done twice and cross-checked.

Cotinine assay

The salivary sample was analysed at a single hospital laboratory, using a double antibody radioimmunoassay, according to the method described by LANGONE and VAN VUNAKIS [17], and adapted for the determination of cotinine from saliva according to COULTAS *et al.* [9]. The rabbit antiserum was supplied by H. Van Vunakis of Brandeis University. A total of 1.0 ml of undiluted saliva was used for the assay, and the range of measurement from the standard curve was 0.1–2.0 $ng \cdot ml^{-1}$ of cotinine. The interassay coefficients of variation for the 0.6, 0.25 and 2.0 $ng \cdot ml^{-1}$ standards were 4.0, 10.8 and 23.7%, respectively. The laboratory personnel were blinded to the exposure levels of subjects.

Analysis

The dependent variable, salivary cotinine level, was found to be distributed exponentially, consistent with the fact that dose-related serum levels for drugs are often based on first-order kinetics. Therefore, log-transformation was used to normalize cotinine measurement for use in multiple linear regression analyses.

The continuous independent variables were the exposure variables, age and hours outdoors (summed over the previous 3 days). The categorical independent variables were: 1) a weather variable, with three levels according to the months in which subjects were assessed, namely, the coolest two months (April and October), the two months of intermediate temperature (May and September), and the warmest three months (June, July and August); 2) the level of aversion to ETS (four levels described above); and 3) time of sample collection (a.m. or p.m.) [4].

For covariables with ETS exposure (those significantly related to log cotinine at $p<0.05$), mean log cotinine levels for categories were compared using a Bonferroni approach to multiple comparison testing [18], in which each and every pair were statistically compared and adjusted for multiple testing bias. For this analysis, the age variable was broken down into four categories: 20–25, 26–32, 33–38, and 39–44 years old.

Spearman rank correlation coefficients were calculated between the exposure variables and untransformed cotinine. Correlations between the various exposure variables were also examined.

The multiple linear regression analysis was carried out using the SAS general linear models procedure [19]. Different models were based upon different approaches to describing recent ETS exposure using questionnaire responses, and were compared on the basis of the amount of variance of cotinine levels that could be explained by the independent variables selected. The ETS exposure models contrasted included: 1) cumulative exposure (duration (hours exposed to smoke) times intensity of exposure (number of smokers)) *versus* separate duration and intensity covariables; 2) correcting for the approximate half-life of cotinine (1.0 (exposure today) + 0.5 (exposure yesterday) + 0.25 (exposure day before yesterday)) *versus* not correcting for half-life; 3) summing exposure duration and intensity over the previous 3 days *versus* the previous 2 days; and 4) a dichotomous variable of no exposure/any exposure *versus* the continuous expressions of exposure above. The importance of location of exposure was also examined. Covariates from the full model with p-values <0.1 were retained using a backward elimination approach.

Results

A comparison group of four proclaimed smokers had salivary cotinine levels ranging from 95.6–309.1 $ng \cdot ml^{-1}$, indicating that the radioimmunoassay accurately detected the presence of active smoking. Descriptive characteristics, cotinine levels, and exposures of the 248 nonsmoking men and women are given in table 1.

Spearman rank correlation coefficients between the cumulative exposure variable and cotinine concentration were similar for exposure, corrected and not corrected for the half-life of cotinine (0.26 and 0.28, respectively). This comparability of correlation coefficients for corrected and uncorrected exposure variables was not surprising, given that the correlation between these two exposure variables was 0.99. The correlation between duration and intensity of exposure was also high at 0.79.

Table 1. – Exposure and descriptive characteristics of subjects

Variable	Men n=125	Women n=123
Mean age yrs	35 (6)	32 (6)
Age groups n subjects		
20–25 yrs	17	17
26–32 yrs	26	43
33–38 yrs	33	44
39+ yrs	49	19
Mean cotinine ng·ml⁻¹	1.1 (1.6)	1.5 (2.3)
Range	0.1–13.3	0.1–14.7
Exposure n subjects		
Yes	75	87
No	50	36
Exposure by location mean person-hours*		
Work	3.3 (8.7)	6.3 (12.6)
Home	0.2 (1.0)	1.0 (2.1)
Social	1.0 (3.5)	1.5 (5.3)
Vehicle	0.1 (0.5)	0.2 (5.3)
Other	0.1 (0.5)	0.3 (1.4)
Total	4.7 (9.3)	9.3 (14.5)
Mean hours exposed**	2.2 (3.8)	4.4 (5.7)
Mean number of smokers exposed to	2.3 (2.7)	2.9 (3.3)
Aversion to ETS n subjects		
None	5	5
A little	34	37
Moderate	40	42
A lot	46	39
Month category n subjects		
Cool	33	24
Intermediate	63	45
Hot	29	54
Total mean hours spent outdoors	6.6 (4.2)	5.8 (4.6)
Time examined n subjects		
Morning	47	47
Afternoon	78	76

Data are presented as mean and standard deviation in parenthesis. *: cumulative number of smokers × hours exposed, corrected for the half-life of cotinine over 3 days (weights are 1.0, 0.5, 0.25, for exposure on the day of cotinine sampling, the day prior, and 2 days prior, respectively); **: corrected for the half-life of cotinine as above. ETS: environmental tobacco smoke.

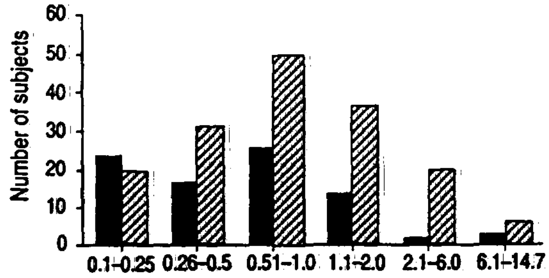

Fig. 1. – Cotinine concentrations in exposed and unexposed subjects. ■: unexposed; ▨: exposed.

Table 2. – Distribution of salivary cotinine levels across categories of variables significantly related to cotinine

Variable	Mean* (SD) log salivary cotinine + 1 Men	Women	Total
Exposure			
Yes**	0.7 (1.0)	0.9 (1.0)	0.8 (1.0)
No	0.2 (1.1)	0.4 (1.1)	0.3 (1.1)
Age Groups			
20–25 yrs	1.1 (1.0)	0.7 (1.1)	0.9 (1.2)
26–32 yrs	0.7 (0.8)	1.1 (1.1)	0.9 (1.0)
33–38 yrs	0.3 (1.2)	0.5 (1.0)	0.4 (1.1)
39+ yrs	0.4 (1.0)	0.8 (1.1)	0.5 (1.0)
Aversion to ETS			
None	1.1 (0.9)	0.6 (0.8)	1.0 (0.9)
A little	0.6 (1.2)	1.1 (1.0)	0.9 (1.1)
Moderate	0.4 (1.0)	0.6 (1.0)	0.5 (1.0)
A lot	0.5 (1.0)	0.6 (1.2)	0.6 (1.1)
Month category			
Cool	0.5 (1.1)	0.4 (0.9)	0.4 (1.0)
Intermediate	0.4 (1.1)	0.7 (1.3)	0.6 (1.2)
Hot	0.7 (1.1)	1.0 (1.0)	0.9 (1.0)

*: transformation used to avoid negative logarithms for cotinine levels <1; **: brackets connecting pairs indicate significant ($p<0.05$) differences between categories, after having accounted for multiple testing bias using Bonferroni comparison tests, for both men and women combined; ETS: environmental tobacco smoke.

It is apparent from Figure 1, that there was considerable overlap in the number of exposed and unexposed subjects within each of six intervals of cotinine concentration. There was, however, on average a greater concentration of cotinine among exposed subjects, evident for both men and women (table 2).

Statistical testing for both genders combined showed differences in mean log cotinine concentrations across categories of the different covariates, and between exposed and non-exposed subjects (table 2). It is evident from the table that trends in the means across categories were not consistent between the two genders. Testing for differences in three day exposure levels between categories of aversion and of temperature failed to reveal differences in log cotinine levels by analysis of variance ($p>0.05$). However, there were significant differences in exposure between age categories for both genders combined. A Bonferroni test showed that the second category (26–32 years old) was significantly different from all of the other categories, with a higher mean level of exposure (11.2 person-hours *versus* 3.0–7.0 person-hours in the other three age categories). Although these differences in exposure may partly explain higher log cotinine levels for ages 26–32 years *versus* the two older categories, the lowest mean person-hours of exposure (3.1) was found in the youngest age category of 20–25 years old, which also had the highest mean cotinine level (1.94 ng·ml⁻¹).

In the multivariate selection procedure, exposure according to questionnaire, age, level of aversion, and time of year were significantly related to log cotinine values ($p<0.05$), and were included in the final regression models. The variables describing gender, hours outdoors, and the time of day (a.m. or p.m.) were not significantly related to log cotinine in any of the regression models examined (all $p>0.39$). Variables describing the interaction between the exposure variables and each of the covariates were not significant.

All regression models explained approximately 15–16% of the variance of log cotinine levels, including those in which duration and intensity were treated as joint (cumulative) or separate variables; the previous two or three days of exposure were examined; the half-life of cotinine was or was not corrected for; or a simple dichotomous exposure variable was used. Estimated regression coefficients, their standard errors and significance levels for the final regression models are given in table 3. Note that regression coefficients are not readily interpretable, due to the necessary log transformation of cotinine, and are presented as a means of comparing the different expressions of exposure.

Table 3. – Regression models for the relationship of environmental tobacco smoke exposure to log salivary cotinine levels in nonsmokers*

Model	Exposure variable**	Regression coefficient	Standard error
1	Cumulative	0.015#	0.004
2	Cumulative, corrected for cotinine half-life	0.020##	0.005
3	Duration, corrected for cotinine half-life	0.015NS	0.017
	Intensity, corrected for cotinine half-life	0.063#	0.027
4	Dichotomous exposure (none/any)	0.466##	0.137

*: the dependent variable is log cotinine; other covariates retained in all models included age, time of year, and aversion to environmental tobacco smoke; **: cumulative=duration × intensity for each location and day of exposure; duration=hours exposed, intensity=number of smokers; all exposures are for the previous 3 days; cotinine half-life correction weight are 1.0, 0.5, 0.25, for exposure on the day of cotinine sampling, the day prior, and 2 days prior respectively; #: $p<0.05$; ##: $p<0.001$; NS: not statistically significant, $p>0.05$.

In a model containing cumulative exposure for each separate place of exposure, significant relationships to log cotinine were found for work exposure ($p<0.01$), and in social exposures ($p<0.04$), whereas exposures at home, in vehicles, or at other places were not significant. This was expected since the great majority of reported exposures occurred at work and in social settings (table 1).

Discussion

Self-reported levels of exposure to environmental tobacco smoke were not strongly related to the level of salivary cotinine, with none of the regression models explaining more than 16% of the variability in log cotinine levels. Thus, little difference was found between standard approaches and present attempts to enhance the ability of questionnaire-derived estimates of ETS exposure to predict salivary cotinine, which included: 1) adjustment of previous days' exposure for the half-life of cotinine; and 2) the use of cumulative exposure, the summed product of exposure intensity multiplied by duration. Similar levels of association have been reported by other investigators, who reported that no more than 23% of the variance of cotinine levels could be explained using multivariate approaches [9, 11, 14]. Exceptions to these studies were the findings of Jarvis *et al.* [5], where parental smoking level explained 44% of the variance of salivary cotinine levels in 569 nonsmoking schoolchildren, possibly because exposure was largely limited to a single place, the home. In the present study, most of the exposures (and their strength of relation to cotinine levels) occurred in the office and social settings, where ETS levels were probably determined by the amount of smoking throughout those sections of a building connected by ventilation systems or large open spaces. Therefore, the actual level of tobacco smoking may not have been as apparent to subjects as in the home, thus explaining the considerable overlap in cotinine concentrations in those reporting exposure with those reporting no exposure (fig. 1), a finding consistent with previous reports [8, 20].

It is important to note that the amount of variance explained may differ between study populations, partly because of differences in the range and distribution of estimated exposures, even though the actual relationship between exposure estimates and cotinine remains the same [21]. Part of the low level of concordance between questionnaire-based estimates of ETS exposure and salivary cotinine is due to inaccuracy in the measurement of cotinine. This may have been higher in the present study, due to the high interassay coefficients of variation observed for our cotinine standards. Cotinine concentrations were, however, concordant with a recent review of studies relating salivary cotinine to ETS exposure [22]; most (96%) of the nonsmokers had cotinine concentrations in the typical range of $<5.0\ ng \cdot ml^{-1}$.

In addition to place of exposure, other determinants of exposure to ETS found in the present study were the level of aversion to ETS, age, and the time of year. An increased level of cotinine for the two lower *versus* two higher levels of aversion was only apparent in men. Higher levels of cotinine were found in the two younger *versus* the two older age categories, similar to findings reported by Cummings *et al.* [14]; however, it was not possible to attribute this finding to differences in exposure, and results differed by gender. The finding that warmer months were associated with higher cotinine levels than cooler months is likely to be spurious, since it is contrary to the findings of previous reports [11, 14], it could not be explained by differences in exposure, it was limited to women, and subjects studied during different months differed as to the place of work (one of two banks).

The modelling approaches in the present study should be re-examined in different settings, to confirm or reject the lack of improvement in the ability of a detailed

questionnaire-derived model to predict cotinine. Our findings do suggest, however, that the cumulative exposure estimate was related more significantly to cotinine ($p<0.001$) than were intensity ($p<0.02$) or duration ($p<0.38$) as exposure covariables. The lack of an independent effect of duration is in contrast to the results of COULTAS *et al.* [11], who found that hours of exposure was the only significant predictor of salivary cotinine.

The present study did not completely account for cumulative exposure, since only the place and each day of exposure could contribute to estimates, not every hour of exposure. A preferable set of questions, as suggested by O'NEILL *et al.* [23], would provide more precise exposure profiles by listing on the questionnaire several lines for each day and each place of potential exposure, with responses to be filled in as: number of smokers for number of hours. Investigators designing questionnaires for studies of acute responses to ETS exposure may gain precision from such an approach.

In the present study, the weak relationship between questionnaire responses and salivary cotinine pertains to recent, not chronic ETS exposures, and thus to studies of the acute health effects of recent exposure. Cotinine as a biological marker of ETS exposure is useful in such studies, given its relatively long half-life and its objective nature, although contradictory findings in the literature argue against considering salivary cotinine to be a gold standard of exposure estimation [24, 25]. Also, from the present study and other similar investigations, it appears that questionnaire assessments of recent ETS exposure are inaccurate, given the low levels of concordance with cotinine despite the use of conceptually better exposure estimates. Further efforts appear necessary to improve the assessment of recent ETS exposure, with investigations aimed at verifying the success of such efforts.

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THE RELIABILITY OF PASSIVE SMOKING HISTORIES REPORTED IN A CASE-CONTROL STUDY OF LUNG CANCER[1]

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Pron, G. E., J. D. Burch (NCIC Epidemiology Unit, U. of Toronto, Toronto, Ontario, Canada M5S 1A8), G. R. Howe, and A. B. Miller. The reliability of passive smoking histories reported in a case-control study of lung cancer. *Am J Epidemiol* 1988;127:267–73.

A test-retest design has been used to examine the reliability of passive smoking histories reported in personal interviews. A total of 117 control subjects initially interviewed in a lung cancer case-control study conducted in metropolitan Toronto, Canada, between 1983 and 1984 were reinterviewed on average six months later. Responses to initial screening questions used to detect a person's exposure to passive smoke were more reliable for residential than for occupational exposure. Respondents also more reliably reported residential exposure to spouse's passive smoke than to the passive smoke of others at home. Quantitative measures of exposure to passive smoke, i.e., number and duration of exposure, were even less reliably reported. Nonsmoking respondents gave the most reliable information. The low reliability of self-reported duration of exposure to passive smoke is consistent with the inability of several studies to detect a significant dose-response relation with lung cancer risk when measures of dose that depend solely on duration are used.

lung neoplasms; occupations; questionnaires; retrospective studies; tobacco smoke pollution

A number of toxic substances, including carcinogens, have been identified in both secondhand (exhaled smoke) and sidestream smoke (smoke emitted from a burning cigarette between active puffs) (1, 2). In particular, sidestream smoke has been shown to contain greater concentrations of some toxic chemicals, e.g., dimethylnitrosamine, naphthalene, benzo(*a*)pyrene, and toluene, than mainstream smoke (smoke actively inhaled) (3). These observations have raised concerns that exposure to passive smoke could be a major contributor to lung cancer among nonsmokers. A number of epidemiologic studies have been conducted with mixed results. A cohort study in Japan (4) and three case-control studies, one in Greece (5) and two in the United States (6, 7), found a significant increased risk of lung cancer among female nonsmokers married to smokers. A recent case-control study (8), also carried out in the United States, suggested that both female smokers and nonsmokers married to active smokers were at increased risk for cancer (all sites combined), although the numbers were insufficient to examine individual cancer sites. The American Cancer Society

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cohort study (9) and a case-control study in California (10) found a nonsignificant relative risk of about 1.2; in another case-control study (11), no difference between cases and controls was found. An analysis that incorporated data on lung cancer risk with passive smoking from 13 different epidemiologic studies estimated a summary relative risk of 1.4 (95 per cent confidence interval 1.19–1.54) (12).

There are a number of possible reasons for these inconsistent results. The use of smoking by a spouse as the only index of passive smoke exposure could lead to a substantial misclassification bias if subjects are exposed at work or at home from household members other than their spouses. The use of hospital controls in case-control studies can be a major source of bias for studies of active smoking, and if passive smoke exposure is associated with diseases that lead to hospitalization, studies of passive smoking would also be biased. Finally, there is the possibility that subjects may provide unreliable information on their passive smoke exposure, since this is obviously a more difficult exposure to measure than that of active smoking.

We have attempted to overcome and assess these difficulties in a case-control study of lung cancer and passive smoking. Lifetime residential and occupational passive smoking histories were requested, population-based controls were used, and a special study was conducted to estimate the reliability of passive smoking histories by means of repeat interviews among a subset of control subjects. This paper reports the results of the reliability study and considers the implications of the results for studies of passive smoking.

Materials and Methods

A test-retest design was employed to examine the reliability of information reported on passive smoking in personal interviews. Respondents in this reliability study were chosen from among controls in a case-control study of lung cancer and passive smoking conducted in metropolitan Toronto. Eligible cases for the lung cancer study consisted of all females newly diagnosed with primary lung cancer in the study area between April 1981 and March 1985. A total of 410 female cases were interviewed together with an equal number of age-matched male subjects randomly selected from lung cancer cases occurring in the same area during the same time period. A total of 780 age- and sex-matched controls. randomly selected from municipal population lists for the same geographic area, were also interviewed. All controls interviewed in 1983 and 1984 were approached six months after their initial interview and were asked if they would agree to be reinterviewed. Of the 147 controls approached for reinterview, 117 (80 per cent) were reinterviewed, 6 (4 per cent) had moved outside the area, and 24 (16 per cent) refused. The study sample consisted of 54 males and 63 females with ages ranging between 33 and 78 years. Among male subjects, 11 (20 per cent) reported on the first interview that they were lifetime nonsmokers, 27 (50 per cent) that they were ex-smokers, and 16 (30 per cent) that they were current smokers. Among female subjects, the numbers were 42 (67 per cent), 13 (21 per cent), and 8 (13 per cent), respectively.

Four specially trained female interviewers conducted the interviews in the homes of the respondents. For each subject, the initial interview and the reinterview were conducted by a different interviewer. This procedure eliminated the possibility that an interviewer could simply record information that she remembered from a previous interview. It was also hoped that the use of different interviewers would ensure that the motivation and participation of the subjects in a reinterview procedure would remain similar.

An identical questionnaire was used on both the initial interview and the reinterview. After active smoking data had been obtained, exposure to passive smoking was ascertained by a series of questions relating to residential and occupational sources of exposure. Residential exposure was deter-

mined by asking an initial screening question, "Have you ever lived in the same household as a regular smoker?" Those who responded positively were asked to identify all such regular smokers and to give the first and last year in which they lived in the same household as the subject. Occupational exposure was also assessed by an initial screening question, "Have you ever worked in a place where you were more or less continually exposed to the tobacco smoke of other people?" Subjects responding positively were then asked to identify each such place of work, the first and last year they worked there, and the number of persons who regularly smoked around them.

The reliability of continuous measures of passive smoke exposure was assessed by the product-moment correlation coefficient. For categorical variables, the kappa statistic, a measure of agreement which is corrected for chance, was used, and for ordered polytomous categorical variables, a weighted kappa statistic (13) was computed. *P* values quoted were two-sided.

RESULTS

The distribution of responses to the question of whether the respondent had ever lived in a house with a regular smoker is shown in table 1 by sex and active smoking status of the respondents themselves and by combinations of sex and smoking status. The agreement generally was good, with all subgroups having similar values of kappa. The corresponding results for exposure to occupational passive smoke are shown in table 2. The prevalence of exposure to passive smoke at work was lower than that of exposure to residential passive smoke, and the kappa values were also lower. The difference between kappas for residential (kappa = 0.66) and occupational (kappa = 0.46) exposure to passive smoke reported by all respondents was not significant (p = 0.09). There appeared to be more variation in kappa values among subgroups for exposure at work than at home, although most differences for the former groups were not statistically significant. Although the kappa value for occupational exposure reports was significantly different (p = 0.04) for male smokers and nonsmokers, the kappa value for reports by male nonsmokers was based on only nine individuals. The highest kappa value for occupational exposure reports was for female nonsmokers, and the highest kappa value for residential exposure reports was for male nonsmokers.

Passive smoking exposures, classified by the number of residential smokers and number of job sites reported, are shown in

TABLE 1

Reliability of response to question on exposure to residential passive smoke, by sex and smoking status of control respondents, lung cancer case-control study, Toronto, Canada, 1983–1984

Sex and smoking status*	First interview/second interview responses					Kappa	Standard error
	Yes/yes (n)	Yes/no (n)	No/yes (n)	No/no (n)	Total		
Both sexes	84	10	4	19	117	0.66	0.084
Females							
All	46	4	4	9	63	0.61	0.124
Never smokers	28	3	2	7	40	0.66	0.141
Ever smokers	18	1	2	2	23	0.50	0.250
Males							
All	38	6	0	10	54	0.70	0.110
Never smokers	6	1	0	2	9	0.73	0.247
Ever smokers	32	5	0	8	45	0.69	0.123

* Subjects in this and subsequent tables were classified as never smokers if they had reported never smoking tobacco products on both interviews and ever smokers if they had reported smoking tobacco products on either or both interviews.

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TABLE 2

Reliability of response to question on exposure to occupational passive smoke, by sex and smoking status of control respondents, lung cancer case-control study, Toronto, Canada, 1983–1984

Sex and smoking status	First interview/second interview responses*					Kappa	Standard error
	Yes/yes (n)	Yes/no (n)	No/yes (n)	No/no (n)	Total		
Both sexes	39	15	16	45	115	0.46	0.083
Females							
All	18	8	6	30	62	0.53	0.109
Never smokers	12	5	2	20	39	0.63	0.126
Ever smokers	6	3	4	10	23	0.37	0.195
Males							
All	21	7	10	15	53	0.35	0.128
Never smokers	3	1	4	1	9	−0.05	0.262
Ever smokers	18	6	6	14	44	0.45	0.135

* The responses of one male (smoker) subject who had reported that he did not know if he had been exposed to occupational passive smoke and of one female (never smoker) subject who had never worked were omitted from this table.

table 3. Kappa values estimated from subgroups of the respondents are summarized in table 4 for these variables. Again, kappa values for occupational exposure were generally lower than those for residential exposure. There appears to be more variability among subgroups from this analysis as compared with tables 1 and 2, reflecting the finer subdivision of the data. In particular, for occupational exposure, there was a statistically significant difference for kappa for female smokers and nonsmokers ($p < 0.01$). Results for residential exposures classified by the relationship of the smoker to the respondent are shown in table 5. The reliability of reported exposure to spouse's smoke was high for both sexes. Exposure to maternal smoke was more reliably reported than exposures to smoke of the father, siblings, children, and other relatives. A similar pattern was observed when respondents were analyzed by sex and smoking status (not shown).

In an attempt to further quantify exposure, duration was calculated for residential exposure (table 6). It should be noted that for some relationships, not all reports were necessarily independent, i.e., one person could report more than one other relative, although this lack of independence should not materially bias the estimate of the correlation coefficient. The kappa value (kappa = 0.52, standard error = 0.088) for the reports of duration of passive smoking was similar to the Pearson correlation coefficient (r = 0.45, 95 per cent confidence interval 0.29–0.58). Correlation coefficients for the reported durations of exposure to passive smoke were generally similar for the various resident smokers. The correla-

TABLE 3

Distribution of reports on the number of resident smokers and job sites from passive smoking histories by control subjects, lung cancer case-control study, Toronto, Canada, 1983–1984*

No. of resident smokers

Second interview	First interview 0	1	2	3+	Total
0	19	7	1	2	29
1	2	15	18	10	45
2	1	5	5	6	17
3+	1	4	3	18	26
Total	23	31	27	36	117

Weighted kappa = 0.55
Standard error = 0.071

No. of job sites

Second interview	First interview 0	1	2	3+	Total
0	45	10	4	2	61
1	9	16	6	1	32
2	4	7	1	1	13
3+	3	3	0	4	10
Total	61	36	11	8	116

Weighted kappa = 0.37
Standard error = 0.101

* Resident smoker is any person the respondent reported living with who was a regular smoker.

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TABLE 4

Reliability of reports on the number of resident smokers and job sites, by sex and smoking status of control respondents, lung cancer case-control study, Toronto, Canada, 1983–1984

Sex and smoking status	No. of resident smokers reported		No. of job sites reported	
	Weighted kappa	Standard error	Weighted kappa	Standard error
Both sexes	0.55	0.071	0.37	0.101
Females				
All	0.52	0.098	0.54	0.126
Never smokers	0.60	0.094	0.76	0.095
Ever smokers	0.37	0.222	0.18	0.183
Males				
All	0.57	0.101	0.21	0.153
Never smokers	0.81	0.147	−0.08	0.296
Ever smokers	0.52	0.116	0.26	0.169

TABLE 5

Reliability of the types of reported resident smokers, by their relationship to the control respondents, lung cancer case-control study, Toronto, Canada, 1983–1984*

Relationship	First interview/second interview responses					Kappa	Standard error
	Yes/yes (n)	Yes/no (n)	No/yes (n)	No/no (n)	Total		
Wife	22	1	2	28	53	0.89	0.064
Husband	37	1	2	21	61	0.89	0.059
Children	8	17	10	82	117	0.24	0.106
Mother	9	4	1	103	117	0.76	0.103
Father	33	27	6	51	117	0.44	0.077
Sibling	9	6	5	97	117	0.57	0.117
Other (relatives)†	2	7	7	101	117	0.16	0.137
Other (nonrelatives)‡	1	13	6	97	117	0.02	0.093

* The responses for exposure to the tobacco smoke of spouses were restricted to ever married subjects—61 of 63 females and 53 of 54 males.

† Other (relatives) includes grandfather, stepfather, father-in-law, son-in-law, brother-in-law, mother-in-law, uncle, and aunt.

‡ Other (nonrelatives) includes boarder, friend, residence roommate, persons in armed forces, rooming house dents, and landlady.

TABLE 6

Reliability of the duration of exposure to residential passive smoke reported for different resident smokers by control subjects, lung cancer case-control study, Toronto, Canada, 1983–1984

Type of resident smoker	Interviews		No. of paired reports	Correlation coefficient	95% confidence interval
	First	Second			
All	24*	21	115	0.45	0.29–0.58
Spouse	27	22	58	0.25	−0.01–0.48
Wife	26	21	22	0.37	−0.06–0.68
Husband	27	22	36	0.20	−0.14–0.50
Parent	25	23	40	0.48	0.20–0.69
Mother	24	22	8	0.69	−0.03–0.94
Father	25	24	32	0.46	0.13–0.70
Other relatives†	13	10	17	0.59	0.15–0.83

* Mean duration (years) of exposure.

† Other relatives include son, daughter, brother-in-law, sister, brother, and uncle.

tion coefficient for the duration of exposure to maternal passive smoke, although higher than for other resident smokers, was based on only eight reports. Reliability for the reported durations of exposure did not vary substantially when the reports were analyzed by the sex and smoking status of the respondent. Correlation coefficients were, however, higher for reports by nonsmoking respondents (table 7). In general, the reliability of this measure of extent of exposure is noticeably lower than the reliability of the qualitative responses shown in table 5.

Discussion

To our knowledge, this is the first study to assess the reliability of information reported on passive smoke exposures in personal interviews. Test-retest estimates of reliability suggest that misclassification of such exposures may be extensive. Responses to an initial screening question used to detect a person's exposure to passive smoke were more reliable for exposure at home than at work. Exposure to spouse's smoke was more reliably reported than exposure to smoke of various other residents in subsequent residential histories. Quantitative measures of exposure to passive smoke, i.e., number and duration of exposures, were even less reliably reported. Generally, nonsmokers gave more reliable information on all measures of passive smoke than smokers. It is of interest to note that for active smoking (details not presented), respondents in this study reliably reported the occurrence (kappa = 0.91, standard error = 0.038) and duration ($r = 0.84$, 95 per cent confidence interval 0.74–0.91) of their own cigarette smoking habits, and the difference between correlation coefficients for active and passive smoking duration was statistically significant ($p < 0.001$) (14).

It is relevant that reliability is a measure of repeatability and not validity, and even if results were completely reliable, there would be no guarantee against misclassification bias in epidemiologic studies. Previous studies (4–6), however, that have obtained risk estimates for lung cancer around 2.0, using exposure to spouse's smoke as the index of exposure, appear to be credible, since that measure of exposure is reliably reported. Nevertheless, the amount of misclassification bias that could result from the degree of unreliability measured in this study for spouse's passive smoke cannot account for the risk estimates for lung cancer around 1.0 found in other studies (9, 10).

To date, studies investigating dose-response relations between exposure to passive smoke and subsequent incidence of lung cancer have had discrepant results. Comparisons between study results are complicated because various measures of intensity or duration, or both, have been employed as indices of dose for different passive smoke exposures, e.g., to smoke of the spouse and to that of the parent. Sig-

TABLE 7

Reliability of the duration of exposure to residential passive smoke, by sex and smoking status of control subjects, lung cancer case-control study, Toronto, Canada, 1983–1984

Sex and smoking status	No. of paired reports*	Correlation coefficient	95% confidence interval
Both sexes	115	0.45	0.29–0.58
Females			
All	62	0.46	0.24–0.64
Never smokers	41	0.54	0.28–0.73
Ever smokers	21	0.31	−0.14–0.65
Males			
All	53	0.44	0.19–0.63
Never smokers	12	0.62	0.07–0.88
Ever smokers	41	0.36	0.06–0.60

* All resident smokers.

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nificant dose-response relations have only been observed in studies that have primarily employed intensity measures of dose, i.e., number of cigarettes per day (4), or intensity and duration measures of dose, i.e., pack-years (6), total number of cigarettes (7), or number of cigarettes over the years of marriage (5). Duration-related measures of dose, i.e., years lived with a smoker (11), or years of exposure to spouse's smoke (7), did not result in significant dose-response relations. The low reliability of durations of exposure to passive smoke reported in this study suggests that it would be difficult to detect significant lose-response relations with measures of dose that depend solely on duration.

The results of this study suggest that improvements in the reliability of measurement of exposures to passive smoke are needed for future studies. It should be noted that when improvements in reliability are impossible, increasing sample size is an alternate strategy to deal with the effects of random error associated with exposure status on risk estimates. Passive smoking appears to be a complex experience, although inconsistent responses by some respondents in this study may be partly attributable to the open-ended format of questions used to obtain information on exposures. An alternate method to measure passive smoking which would use specific probes for various exposures, e.g., to smoke of a ›ouse, parent, or sibling, to aid subjects' recall of their exposures may result in more reliable information. The unreliability of duration measures of dose used in this study, e.g., years lived with a person who smoked, suggests that other measures of dose should be employed for the study of exposures to passive smoke.

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SECTION 3

ETS AND LUNG CANCER

CHEMICAL MARKERS

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ARTEFACTS IN DETERMINING THE VAPOUR-PARTICULATE PHASE DISTRIBUTION OF ENVIRONMENTAL TOBACCO SMOKE NICOTINE

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ABSTRACT

Nicotine in environmental tobacco smoke (ETS) resides predominantly in the aerosol vapour phase as a result of evaporation from the particles of sidestream smoke. In true ETS (*i.e.*, not concentrated or fresh sidestream smoke), the fraction of nicotine associated with the aerosol particulate phase is quite small, typically less than 5% of the total. Recently, some investigators have collected nicotine with sampling systems employing sorbent resin cartridges downstream from glass-fibre filters, and have attributed the nicotine retained on the glass-fibre filters to the particulate phase. The data reported here demonstrate that phase distributions and dynamics determined using glass-fibre filters are due to sampling system artefacts. Glass-fibre filters collect virtually all nicotine (vapour- and particulate-phase) at relatively short sampling intervals (1-2 min). The percentage of total nicotine trapped on the filter decreases with increasing sampling time. Using such a system, only that amount of total nicotine which exceeds the adsorptive capacity of the filter will break through and be collected by the sorbent medium. Attributing ETS particulate-phase nicotine to the quantity collected on a glass-fibre filter leads to erroneous conclusions regarding vapour-particulate phase dynamics.

Keywords: Aerosol, environmental tobacco smoke (ETS), nicotine, particles, vapour

INTRODUCTION

It is now firmly established that nicotine in environmental tobacco smoke (ETS) resides predominantly in the aerosol vapour phase as a result of evaporation from the particles of sidestream smoke. This finding was first presented in 1985 (1), published the following year (2), and verified by other researchers (3). During these investigations, numerous independent analytical techniques were used to reach this conclusion, including: denuder sampling (2,3) and denuder, filter and sorbent bed sampling in concert with electrostatic precipitation and real-time detection by atmospheric pressure chemical ionization (APCI) mass spectrometry (2).

The fraction of true ETS (*i.e.*, not concentrated or fresh sidestream smoke) nicotine associated with the aerosol particulate phase is quite small, typically less than 5% of the total (2-6). However, there are indications that the vapour-particulate distribution of ETS nicotine can be perturbed by exaggerated smoke concentrations (4) and by intense UV irradiation of the smoke (6). For example, with test chamber nicotine concentrations of *ca.* 100 $\mu g\ m^{-3}$, 99% of total nicotine is in the gas phase while at chamber concentrations of *ca.* 500 $\mu g\ m^{-3}$, 92% of the total is gas-phase nicotine (4). It is not known to what extent, if any, these perturbations may affect nicotine measurements in typical, real-world environments; however, ETS nicotine concentrations above 100 $\mu g\ m^{-3}$ are rarely encountered (7). In addition, the absolute certainty of these percentages is debatable due to the dynamic nature of the ETS aerosol and due to the fact that nicotine is highly adsorptive and is a semivolatile compound which tends to evaporate readily from aerosol particles. As such, it is not easy to measure phase distribution precisely because it is easily disturbed by the method of measurement.

Recently, Neurath *et al.* (8,9) and Esquier

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and Hée (10) reported concentrations and time variations in the particulate-phase distribution of ETS nicotine that are contrary to the established theory of dynamics. These investigators collected nicotine with sampling systems employing sorbent resin cartridges downstream from glass-fibre filters and attributed the nicotine retained on the glass-fibre filters to the particulate phase. The adsorption of nicotine on glass-fibre filters was examined in the investigation reported here and the findings explain the deviation in apparent nicotine phase residence obtained using glass-fibre filters. Consequently, recent results concerning phase dynamics derived from nicotine collected on glass-fibre filters (8-10) are in error.

Determining which phase of ETS aerosol contains nicotine is not merely an academic pursuit. The phase residence is important in studying ETS exposure in that it may impact the mechanism and magnitude of nicotine retention by non-smokers. Also, there are significant consequences for predicting decay mechanisms of nicotine in indoor spaces as well as in understanding the variability between nicotine and other components of ETS and, ultimately, in determining the usefulness of nicotine as an ETS marker.

MATERIALS AND METHODS

Environmental Chamber

Test atmospheres were generated in an 18-m^3 environmentally-controlled test chamber (11). The chamber was ventilated continuously at eight air changes per hour. Temperature and relative humidity (RH) of the chamber air were nominally 20°C and 58%, respectively. Actual temperatures fluctuated between 20 and 22°C due to the presence of one person throughout the experiments.

Nicotine Vapour Generation

Nicotine vapour was generated using a model 585A Precision Gas Standards Generator (Kin-Tek Laboratories, Inc., Texas City, TX). Nicotine vapour was swept by dry (0% RH) nitrogen from a sealed, thermostated (100°C) glass reservoir containing a diffusion tube of nicotine liquid. The Teflon tubing carrying the nicotine vapour was routed into the test chamber where the gas stream was diluted with humidified chamber air to achieve target nicotine concentrations. The resulting humidity of the mixed vapour streams was 50% RH. To ensure a particle-free atmosphere, both gas streams were double-filtered, first with a Fluoropore Teflon membrane filter (37-mm diameter, 1.0-µm pore size, FALP 03700, Millipore Corp., Bedford, MA) and followed by a laminated glass-fibre filter (part no. 6004300, Gelman Sciences, Ann Arbor, MI). Steady-state nicotine concentrations in the gas stream were achieved by allowing the system to equilibrate overnight ($\geq$ 18 hrs) prior to all sample collections.

The lack of particles in the gas stream being sampled was verified with a condensation nucleus counter (CNC, model no. 3020, TSI Inc., St. Paul, MN).

Nicotine Collection

Sampling systems used for trapping nicotine consisted of an XAD-4 sorbent tube (226-30-11-04, SKC Inc., Eighty-Four, PA) connected downstream from either a borosilicate glass-fibre filter (T60A20, Pallflex Co., Putnam, CT) or a Teflon membrane filter (Fluoropore FALP 03700). In both cases, a bisulfate-treated glass-fibre filter (Pallflex TX40HI20WW) was positioned downstream from the XAD-4 sorbent tube to ensure that all nicotine was collected (12). In all cases, connecting tubing was kept to an absolute minimum. Flow through each sample train was maintained at 2.2 L min^{-1} using an electronic mass flow controller (Teledyne Hastings-Raydist, Hampton, VA). A second mass flow controller was adjusted to dilute the nicotine vapour from the gas standards generator to a level comparable to that employed by Neurath *et al.* (9); the target concentration was 1500 µg m^{-3}. Likewise, the untreated glass-fibre filter (Pallflex T60A20) and flow rate through the sample train (2.2 L min^{-1}) are the same as used by Neurath *et al.* (9). The entire experimental set-up is represented schematically in Figure 1.

Five replicates were obtained with each sampling system at each of 1-, 2-, 3-, 5-, and 10-min collection times. All samples were extracted and analyzed within 48 hrs of sample collection.

Analytical Methods

Nicotine collected on XAD-4, untreated glass-fibre filters (Pallflex), and Teflon filters (Fluoropore) was extracted in triethylamine-modified ethyl acetate followed by gas chromatography with *N*-thermionic detection using quinoline as internal standard (13-16).

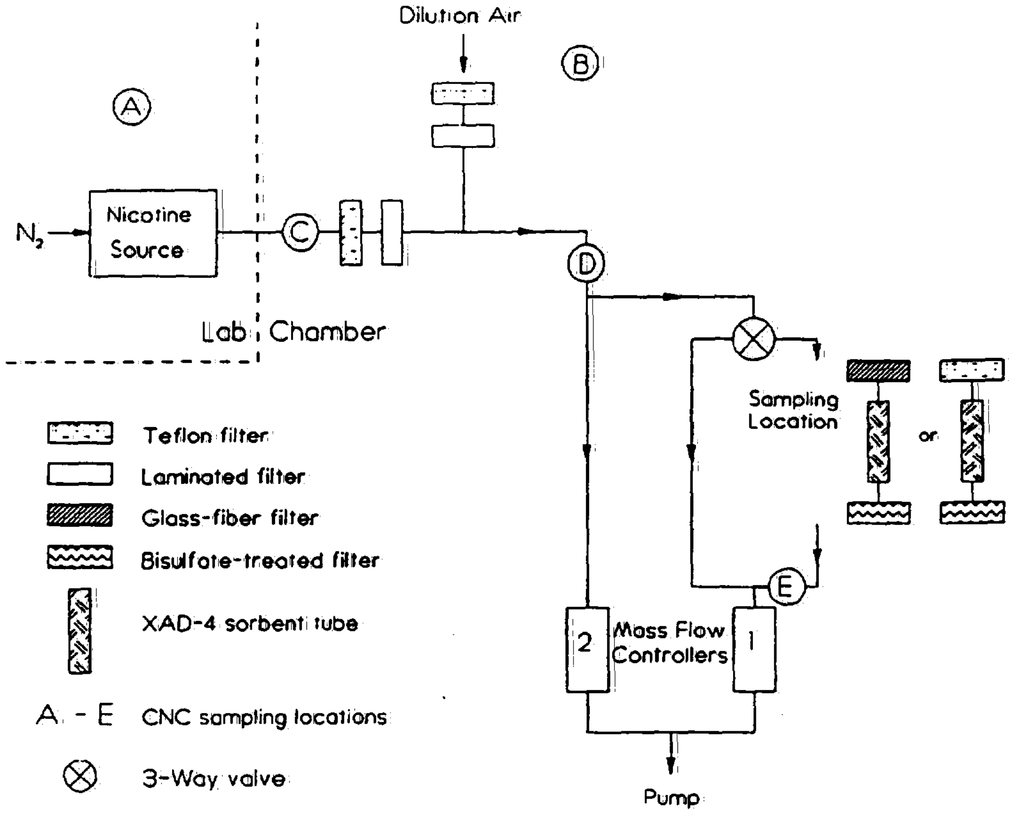

Figure 1. Schematic diagram of experimental set-up. 3-Way valve is used to direct nicotine vapour either through sample train or to bypass.

Nicotine trapped on bisulfate-treated glass-fibre filters was extracted in ethanol-modified sodium hydroxide with back extraction into ammoniated heptane (12). Gas chromatographic conditions were the same as for the XAD procedure except that *N*-ethylnornicotine was used as internal standard.

In preliminary experiments, it was confirmed that nicotine recovery from the untreated glass-fibre filter was equivalent when using either the direct ethyl acetate extraction or the more cumbersome sodium hydroxide/heptane partitioning.

RESULTS AND DISCUSSION

Particle concentrations at each sampling point denoted in Figure 1 are listed in Table 1.

Table 1. Particle concentrations at sampling points illustrated in Figure 1 as measured by a condensation nucleus counter (CNC).

Sample Location Descriptor	Sampling Position Noted in Figure 1	CNC Response (particles cm^{-3})*
Laboratory air	A	7.3×10^3
Chamber air	B	
experiment start		4.2×10^1
experiment end		1.3×10^3
Before filters	C	0.03×10^0
After filters/before sample train	D	0.00×10^0
After sample train	E	0.00×10^0

*Data presentation reflects reading and range values as determined by the CNC.

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Table 2: Vapour-phase nicotine retained on filter collection media as a function of sampling duration.

Sampling Duration (min)	Pallflex (glass-fibre) filter (± SE, n=5)		Fluoropore (Teflon) filter (± SE, n=5)	
	µg filter^{-1}	% of total	µg filter^{-1}	% of total
1	3.46 ± 0.02	97.3 ± 0.2	0.14 ± 0.01	4.9 ± 0.3
2	5.15 ± 0.20	75.3 ± 2.0	0.16 ± 0.01	2.4 ± 0.2
3	6.09 ± 0.17	62.1 ± 1.6	0.17 ± 0.01	1.8 ± 0.1
5	6.20 ± 0.34	38.2 ± 1.6	0.27 ± 0.03	1.5 ± 0.2
10	6.95 ± 0.36	20.3 ± 0.5	0.28 ± 0.06	0.8 ± 0.1

The complete absence of any measurable particle concentration directly upstream from the sample collection assembly verifies the presence of only vapour-phase nicotine. The average concentration (± SE, n=50) of nicotine vapour being sampled was 1513 µg m^{-3} (± 18).

Results of nicotine determination on the two filter collection media are presented in Table 2. As can be seen, the untreated glass-fibre filter has a substantial capacity for adsorbing nicotine. Shown in Figure 2 is a plot of nicotine mass adsorbed on the untreated glass-fibre filter (Pallflex) as a function of sample duration showing an adsorptive capacity approaching 7 µg under the atmospheric conditions used. Although there appears to be an increasing trend still present between five and 10 min, these two values are not statistically different. Also shown in Figure 2 is the minimal quantity of vapour-phase nicotine trapped by Teflon filters under identical conditions.

The filter/XAD-4 combinations trapped

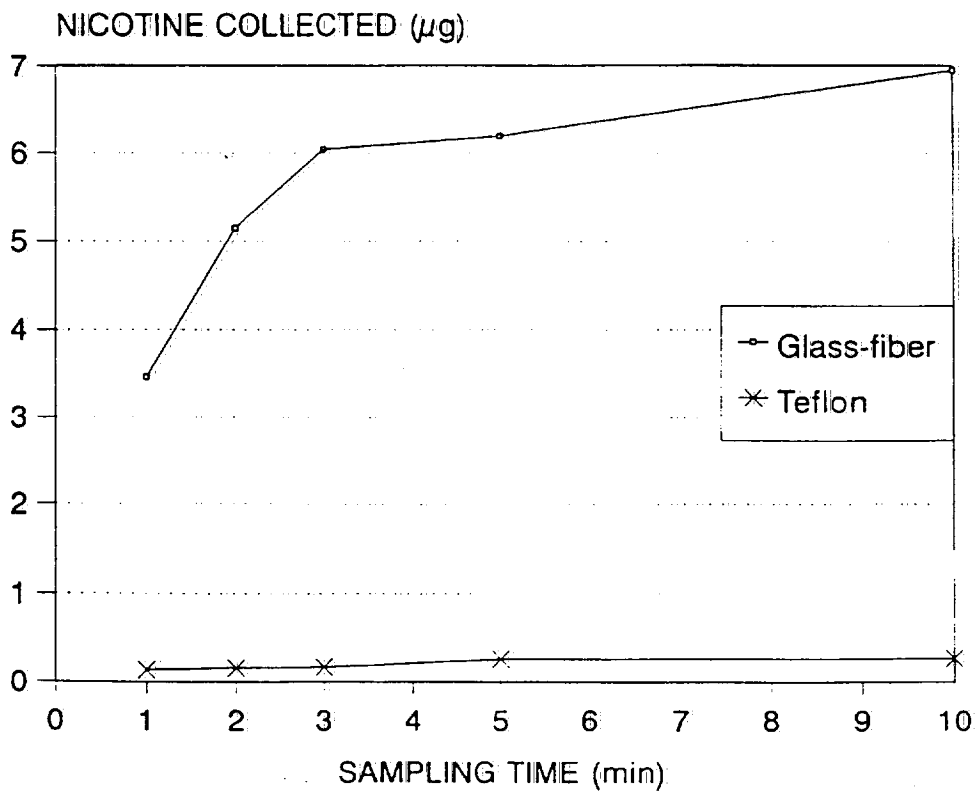

Figure 2. Vapour-phase nicotine retained on borosilicate glass-fibre (Pallflex T60A20) and Teflon (Fluoropore) filters as a function of sampling duration.

>99% of the total vapour-phase nicotine. The average (± SE, n=50) nicotine breaking through onto the bisulfate-treated filter was 0.8% (± 0.2).

Expressing the quantities of nicotine retained on the first filter as a percentage of the total amount collected by each sample train (Figure 3) shows that with only 1-min sampling, nearly all of the vapour-phase nicotine (>97%) is trapped on the glass-fibre filter. In contrast, nicotine collected on the Teflon filter ranges from *ca.* 5 to 1% of the total at collection times ranging from 1 to 10 min.

The phenomenon illustrated by these data is the same as that observed by Neurath *et al.* (8,9) and Esquier and Hée (10). However, to attribute these results to the presence and collection of particulate-phase nicotine, two assumptions must be valid. First, the glass-fibre filter used must trap only particulate-phase nicotine and allow vapour-phase nicotine to pass unhindered. Second, any collected nicotine associated with the particulate matter must be stable and will not be evaporated from the particles (and thus lost to the backup sorbent resin) under the influence of the continuous air stream being sampled.

The data presented here demonstrate that the first assumption is not valid for glass-fibre filters. Assuming that all nicotine retained by a glass-fibre filter derives from particulate-phase nicotine will result in gross overestimation of the nicotine associated with that phase. The degree of overestimation is most pronounced with very short sampling times of only a few minutes.

This phenomenon had already been demonstrated in the literature for Cambridge (glass-fibre) filters (2). Other data in the literature show *ca.* 1 µg of nicotine adsorbed completely from solution onto the inside surfaces of borosilicate glass vials (14). It appears reasonable, therefore, that a borosilicate glass-fibre filter with several orders of magnitude more surface area than a glass vial could adsorb at least one order of magnitude more nicotine from air. Our results indicate that the glass-fibre filters used here and elsewhere (8,9) are an efficient collection medium for vapour-phase nicotine at sampling times of a few minutes or less. We have not attempted to show what dependence the nicotine adsorption on glass-fibre filters may have on sampling time, air flow rate, temperature, RH, or nicotine concentration.

Likewise, there are data in the literature

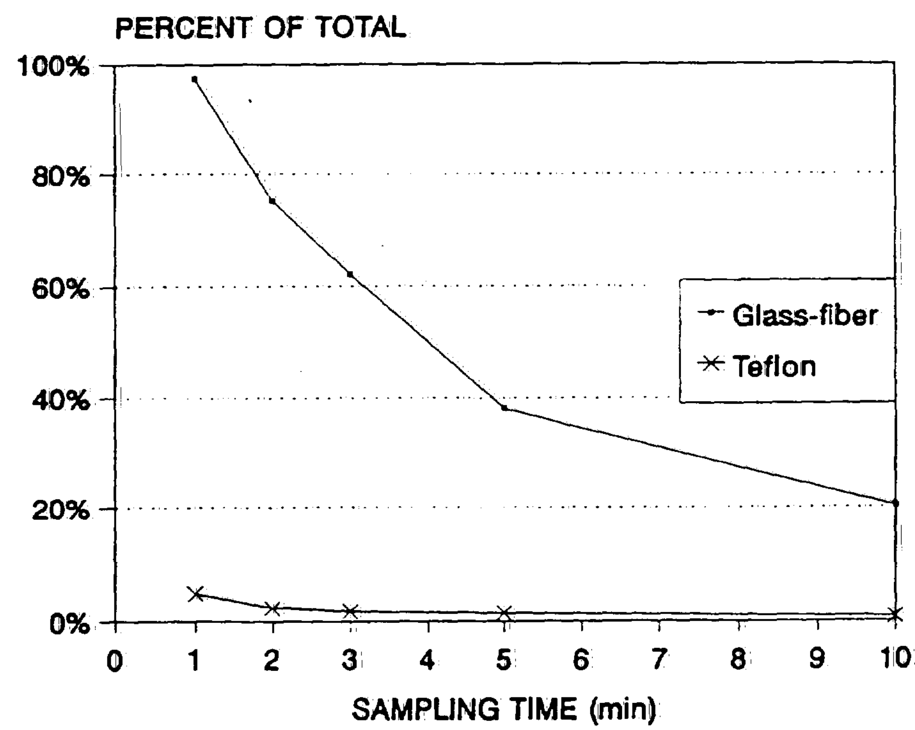

Figure 3. Percentage of total vapour-phase nicotine trapped by borosilicate glass-fibre (Pallflex T60A20) and Teflon (Fluoropore) filters as a function of sampling duration.

which negate the second assumption outlined above. In an intercomparison study of sampling techniques for nicotine (17), untreated glass-fibre filters used to collect particulate-phase nicotine passing through denuders yielded results which were biased low compared to acid-treated filters used after denuders. Esquier and Hée (10) also correctly identified the problem of nicotine evaporation from an untreated filter during sampling.

Any nicotine truly present in the aerosol particulate phase would be trapped initially by glass-fibre filters. However, this minute quantity will be totally obscured by simultaneous collection of nicotine vapour. Combined with the almost certain loss of some true particulate-phase nicotine due to evaporation and elution from the filters, the data obtained from use of such filter systems are completely uninterpretable with respect to the vapour-particulate phase dynamics of ETS nicotine.

CONCLUSION

Use of combined filter/sorbent sampling systems for studying the distribution and dynamics of nicotine between aerosol phases is inappropriate. Borosilicate glass-fibre filters collect virtually all nicotine (vapour- and particulate-phase) at relatively short sampling intervals (1-2 min). Using such a system, only that amount of total nicotine which exceeds the adsorptive capacity of the filter will break through and be collected by the sorbent medium. Attributing ETS particulate-phase nicotine to the quantity collected on the glass-fibre filter leads to erroneous conclusions.

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PROBLEMS WITH THE USE OF NICOTINE AS A PREDICTIVE ENVIRONMENTAL TOBACCO SMOKE MARKER

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A series of experiments was performed to evaluate the utility of nicotine as an environmental tobacco smoke (ETS) marker. Two University of Kentucky reference cigarettes (1R4F) were smoked in an 18-m^3 environmental chamber. Air exchange rates within the chamber were varied from 0-4 air changes per hour, and the concentrations of numerous ETS components were monitored for up to six hours after smoking. Under most ventilation conditions, nicotine initially decayed more rapidly than other ETS constituents; however, as sampling time was extended, nicotine decayed more slowly. The change in nicotine decay rate can lead to overestimation of ETS exposure when nicotine is used as the sole ETS marker. Confirmatory results obtained from other field and chamber studies are also presented.

INTRODUCTION

The utility of nicotine as an environmental tobacco smoke (ETS) marker has been questioned by a number of investigators (1-3). Some have simply stated that it is a poor marker (1), while others have suggested that nicotine may underestimate ETS exposure (2,3) by as much as an order of magnitude.

The National Research Council (NRC) recommended that any chosen ETS marker should be present in a consistent ratio to ETS components of interest (4). To attain this criterion, the marker should possess the same decay characteristics as the component of interest.

If an ETS component is not generated or eliminated by chemical reaction, or does not interact with environmental surfaces, then it should demonstrate a first order decay with a rate constant proportional to the air exchange rate in a microenvironment. Previous studies have shown that nicotine does not undergo first order decay in microenvironments (2,3).

This present work was performed to systematically evaluate the effect of nicotine's unique decay on its ratio to other ETS components. Due to nicotine's different decay rate, both ventilation rate and sampling time would be expected to exert an effect on the ratio of nicotine to other ETS constituents. Therefore, ratios were determined at air exchange rates of 0, 0.5, 1, 2 and 4 air changes per hour (ACH), and time-weighted-average concentrations of selected analytes were determined for 0-30, 0-60, 0-120, 0-240, and 0-360 minutes following the smoking of 2 University of Kentucky reference cigarettes (1R4F). The results obtained in the chamber were then related to results obtained previously in field and chamber studies.

EXPERIMENTAL

Chamber Studies

All ETS decay experiments were performed in an 18-m^3 environmental chamber described elsewhere (5). Three to five replicate experiments were performed at 0, 0.5, 1, 2, and 4 ACH. Real-time nicotine concentrations were monitored with a SCIEX TAGA 6000 tandem mass spectrometer (6). Real-time concentrations of carbon monoxide, nitrogen oxides, volatile organic compounds (estimated by FID response), and particle mass concentration were obtained with commercial analyzers described elsewhere (7). Vapor phase nicotine and 3-ethenylpyridine were collected using XAD-4 sorbent tubes and analyzed by gas chromatography with nitrogen phosphorus detection (8). Solanesol, gravimetric respirable suspended particles (RSP), ultraviolet particulate matter (UVPM), and fluorescent particulate matter (FPM) were collected on Fluoropore filters and analyzed as described elsewhere (9,10). Duplicate nicotine and particulate samples were collected over the periods 0-30, 30-60, 60-120, 120-240, and 240-360 minutes, and integrated average concentrations were determined.

Each run during the decay rate studies lasted a total of 384 minutes. The first twelve minutes of the run were used to measure background concentrations of ETS constituents. A smoker then entered the chamber and smoked two University of Kentucky 1R4F cigarettes in 10½ minutes. The two cigarettes were lit at 30-second intervals, and the smoker took one puff on alternating cigarettes at one-minute intervals. Each cigarette lasted for an average of ten puffs. At 24 minutes, the smoker exited the chamber which was subsequently resealed for the final 360 minutes of the experiment.

The effect of residual nicotine on a smokers' clothing was determined from the average of 15 runs performed at various times over a three-month period in the environmental chamber which was operated at 0 ACH. After a twelve-minute background measurement, the smoker entered the chamber and stayed for twelve minutes. The smoker then exited the chamber, and nicotine concentration was monitored for an additional 36 minutes.

Field Studies

Nicotine samples were collected on XAD-4 sorbent tubes and analyzed by the method of Ogden et al. (8). Six-hour samples were collected in a smoker's van and the den of a non-smoker's house. Eight one-hour samples were collected overnight in a B767 aircraft which had completed a flight on which smoking was allowed.

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Particulate samples were collected and analyzed for the aircraft study by the same methods used in the chamber study.

RESULTS AND DISCUSSION

Nicotine Decay

At each of the air exchange rates studied, nicotine initially decayed more rapidly than the other ETS constituents measured. However, at longer times the decay of nicotine slowed, and typically achieved a near-steady state concentration which was higher than the initial background level. On the other hand, constituents such as CO, volatile organic compounds, nitrogen oxides, and particle mass concentration decayed according to first order kinetics with decay rate constants which were proportional to the air exchange rate. The other constituents did decay to initial background levels in a time-scale consistent with normal first order decay.

A plot of nicotine concentration vs. time obtained from the average of five runs performed at 2 ACH is shown in Figure 1. The solid line in this figure is the average nicotine concentration measured in the chamber. The dashed line in the same figure is the nicotine concentration profile which would be predicted by first order decay. The theoretical curve in Figure 1 is representative of the behavior of the other measured ETS constituents. Time-weighted-average (TWA) concentration ratios between nicotine and the other constituents are proportional to the ratio of the areas under the two curves. For a sampling period extending from 0-30 minutes, the ratio of nicotine to analyte will be lower than that predicted by first order kinetics. At about 30 minutes, nicotine decays less rapidly than the other constituents, and for sampling times greater than 60 minutes, the ratio of nicotine to other analytes will become increasingly larger than those predicted by first order decay of constituents. The magnitude of overestimation becomes larger if measurements are started at times long after smoking has occurred. For the example illustrated in Figure 1, the other constituents would have decayed to background at about 180 minutes; but significant concentrations of nicotine are still present in the chamber. In this case, a person exposed to the atmosphere in the chamber would not be exposed to measurable ETS particulate, CO, or volatile organic compounds, but they would have measurable exposure to nicotine.

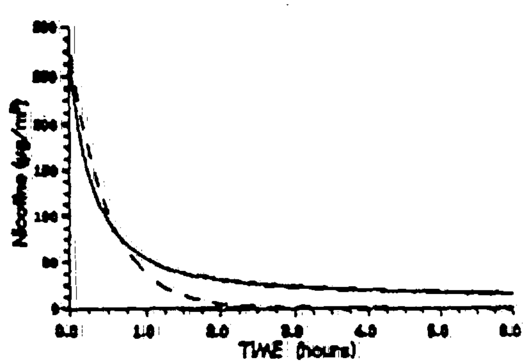

Figure 1. Average of five real-time nicotine concentrations (solid) measured in a controlled environment chamber operated at two ACH. The dashed line represents the concentration predicted by a first order decay mechanism.

Ratios Involving Nicotine

The actual variations of nicotine to RSP and nicotine to FID response ratios as a function of both sampling time and air exchange rate are shown in Figures 2a

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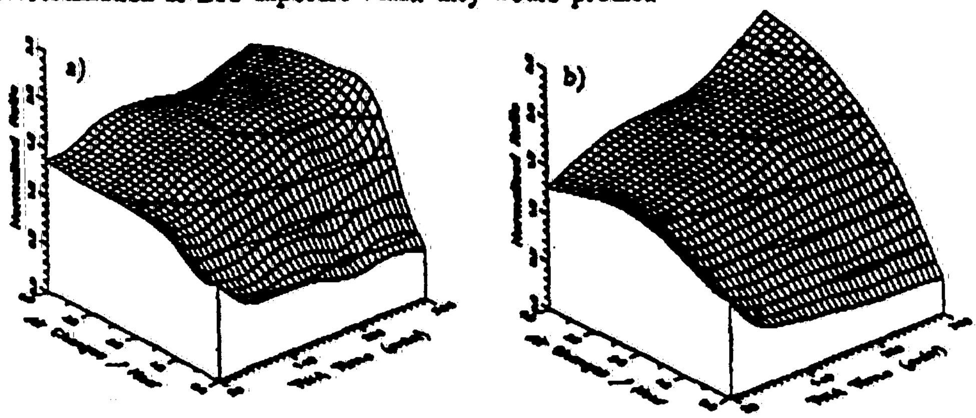
and 2b. Ratios presented in these figures have been normalized to the values obtained for the 0-30 minute sample at 0 ACH. Sampling for increasing periods of time or sampling for a constant period of time at different air exchange rates has a dramatic influence on the observed ratio of nicotine to either vapor or particulate phase ETS components. Furthermore, these figures demonstrate that ratios of nicotine to other ETS components which are determined in chambers operated in static modes are not applicable to other environments because of the large overestimation of ETS exposure which they would predict.



Figures 2a & 2b. Normalized ratios between nicotine and gravimetric RSP (2a) and FID response (2b) as a function of both air exchange rate and sampling time. All values in figure 2a and 2b have been normalized to the ratio at 0 ACH and 30 min (0.108 in 2a & 74.1 in 2b). Lines of constant ratio are drawn at intervals of 0.333 beginning at a ratio of 0.667.

Field Measurements

Results consistent with those obtained in the chamber also have been observed in the field. Background nicotine concentrations in the absence of smoking have been measured in homes, automobiles and aircraft. In addition, nicotine desorption from the clothes and person of a smoker has been observed in the environmental chamber. Results of these studies suggest that the initial rapid decay of nicotine is due to its adsorption on clothing and other surfaces. As atmospheric nicotine is depleted, due to the effects of adsorption and dilution by fresh air, adsorbed nicotine then desorbs, and leads to measurable nicotine concentrations in the absence of smoking.

Figure 3 shows that smokers can be a source of nicotine contributed to the environment even when they are not smoking. This figure shows the average increase in nicotine concentration measured during 15 replicate experiments performed over a three-month period in which a smoker entered the environmental chamber for twelve minutes. The increased nicotine concentration is presumably due to the evolution of nicotine from the smoker's clothes. When the smoker exited the chamber, the nicotine concentration ceased its increase.

A nicotine concentration of 0.09 $\mu g/m^3$ was measured in the den ($\sim$45 m^3) of a non-smoker's house. The sample was obtained two days after smoking had occurred in the room. This background level corresponds to small amounts of residual nicotine desorbing from room furnishings over a long period of time.

Nicotine samples were collected in a van ($\sim$5 m^3) in which smoking regularly

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occurred. The sample was taken overnight and at least four hours after smoking had occurred in the vehicle. The sample was obtained at dashboard level ~40 cm above an open ashtray. A background concentration of 0.126 $\mu g/m^3$ was measured in the vehicle. Once again, this background is not due to the presence of ETS, but instead it comes from nicotine desorbing from the interior of the van and from cigarette butts present in the ashtray.

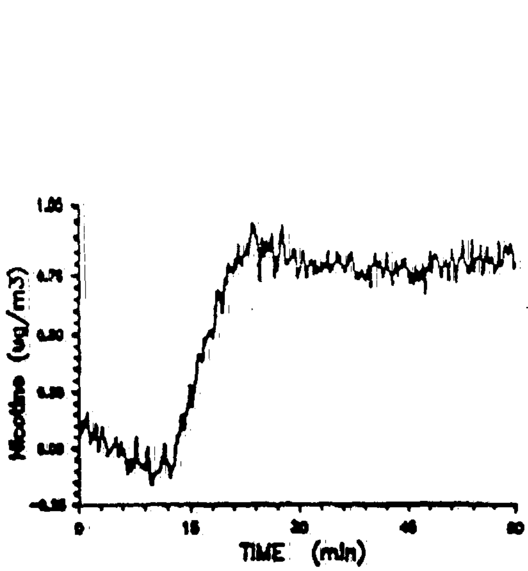

Figure 3. Average increase in nicotine concentration due to the nicotine desorption from a smoker's clothing in a controlled environment test chamber operated at 0 ACH. The smoker was in the chamber for the period 12-24 minutes.

Samples were also collected in a B767 aircraft in which smoking was permitted. The aircraft had returned from a regularly scheduled 4 hour 20 minute flight from Los Angeles, CA to Charlotte, NC. Samples were collected when the plane was on the ground and parked at the gate after all passengers and crew had left the plane. While the samples were collected, the aircraft was served by an auxiliary heating, ventilating and air conditioning system which provided fresh air at 13-26 ACH (11). Smoking was not permitted while the aircraft was at the gate, and none was observed. Samples were obtained in the coach smoking section and seats in the non-smoking border section. A total of 64 cigarette butts were counted in the ashtrays prior to sampling. The results of this investigation are presented in Table I. During time period 4, the crew collected trash, emptied ashtrays during period 5, and vacuumed the cabin during period 7. UVPM measurements obtained from samples taken over the entire 8-hour period showed less than 1.5 $\mu g/m^3$ particulate matter was present which could possibly be attributed to ETS. Once again, the only source of nicotine in the cabin would appear to be nicotine desorbing from interior surfaces and cigarette butts. Measurable nicotine exposure could be expected in the non-smoking boundary section without concurrent exposure to ETS gas or particulate phase material.

Table I. Background nicotine concentrations ($\mu g/m^3$) measured overnight on a B767 aircraft with auxiliary ventilation. Smoking samples (S.) were obtained in the coach smoking section. Non-smoking samples (N.S.) were obtained in coach smoking/nonsmoking border seats.

Sample	Time	Nicotine S.	Nicotine N.S.
1	22:20-23:20	5.2	1.7
2	23:20-00:20	4.9	1.3
3	00:20-01:20	2.8	1.1
4	01:20-02:20	8.7	1.8
5	02:20-03:20	8.0	1.6
6	03:20-04:20	4.7	1.3
7	04:20-05:20	5.2	2.7
8	05:20-06:20	8.2	2.5

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CONCLUSIONS

Nicotine does not fit the NRC criteria for an ETS marker. The ratio of nicotine to other ETS constituents such as RSP are highly variable and dependent on both the air exchange rate at the sampling site and sampling time. Desorption of nicotine from clothing, interior surfaces, and cigarette butts leads to measurable nicotine exposure in the absence of ETS. The findings reported here show that when nicotine is used as the sole marker, it may greatly overestimate ETS exposure.

ACKNOWLEDGEMENTS

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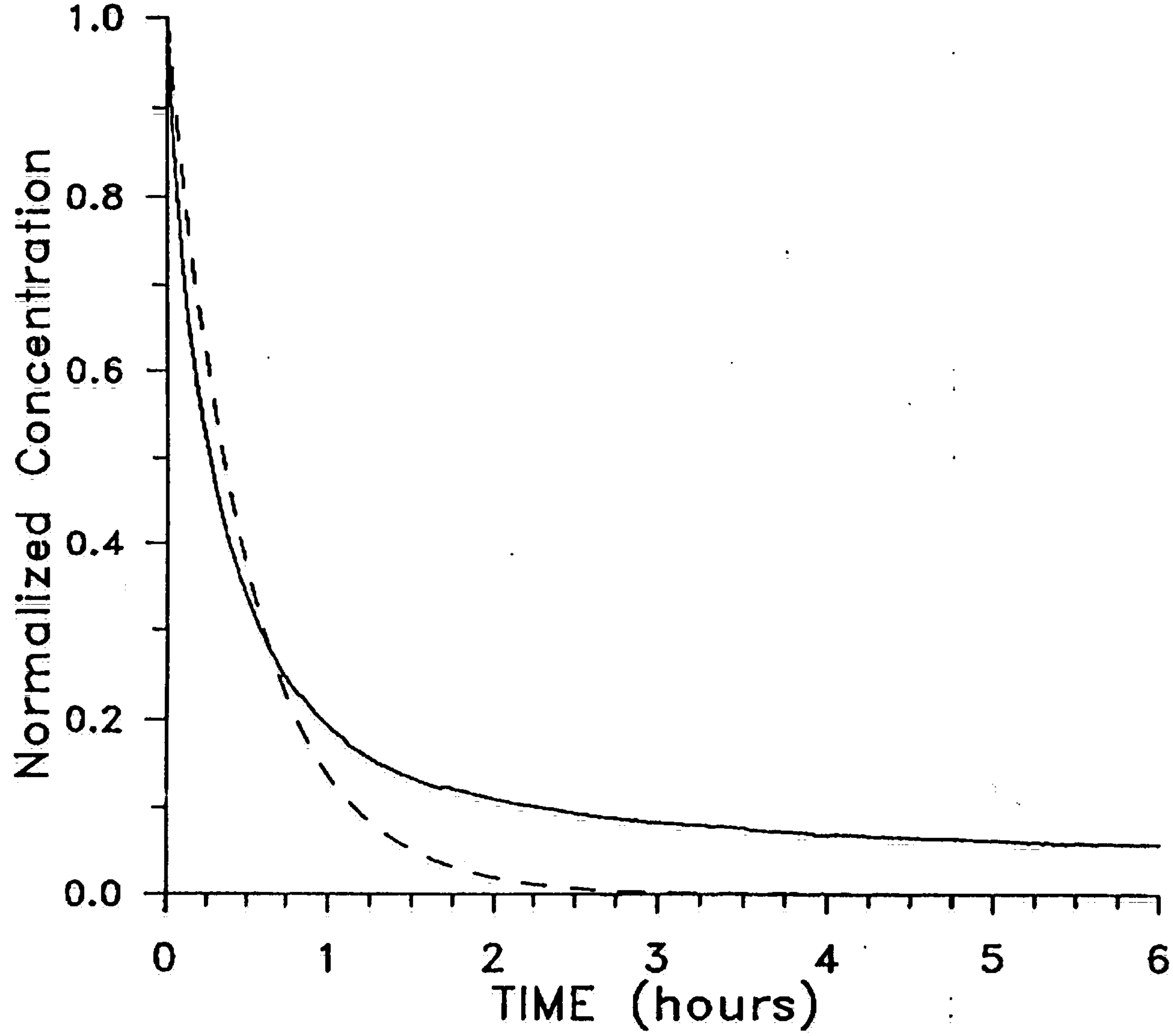

Nelson et al.
Figure 1.

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SECTION 3

ETS AND LUNG CANCER

PERSONAL MONITORS

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ment conditions.

KING POLICY

Restricted Filtration [RF]	Restricted No Treat. [RNT]	Restricted Workstation [RWS]
0.4	0.6	0.1
(0.1-2.1)	(0.1-3.3)	(0.0-0.5)
674	634	573
(575-791)	(533-755)	(488-671)
0.02	0.05	0.01
(0.013-0.047)	(0.025-0.103)	(0.008-0.028)
2[illegible] ± 14.02	5.3 ± 15.32	2.6 ± 14.0
114.[illegible] ± 22.9	19.8 ± 25.1	10.2 ± 22.9
20.8 ± 2.4	3.8 ± 2.6	2.4 ± 2.4
23.9 ± 0.4	23.7 ± 0.4	23.6 ± 0.4
33.4 ± 3.5	28.3 ± 3.9	35.9 ± 3.5
[illegible]07.9 ± 62.4	586.4 ± 67.8	564.9 ± 62.4

for nonsmoking and smoking areas of

KING POLICY

RF Smoking	RNT Nonsmoking	RNT Smoking
1.2	0.4	1.0
(0.7-2.1)	(0.2-0.8)	(0.4-2.2)
676	597	698
(550-644)	(550-647)	(624-781)
0.04	0.05	0.06
(0.030-0.049)	(0.038-0.063)	(0.039-0.079)
126.1 ± 8.8	19.3 ± 9.0	35.6 ± 12.5
236.6 ± 20.0	9.3 ± 20.5	35.6 ± 28.6
4[illegible] ± 3.6	0.3 ± 3.8	10.3 ± 5.2
[illegible] ± 0.2	23.7 ± 0.2	23.8 ± 0.2
33.6 ± 1.1	28.2 ± 1.2	28.7 ± 1.6
503.6 ± 42.0	648.8 ± 43.0	474.5 ± 60.3

ing areas

MULTIPLE MEASURES OF PERSONAL ETS EXPOSURE IN A POPULATION-BASED SURVEY OF NONSMOKING WOMEN IN COLUMBUS, OHIO

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ABSTRACT

Environmental tobacco smoke (ETS) exposure was assessed continuously over a one-week period in a group of 96 nonsmoking, married women in Columbus, Ohio. Markers used to estimate ETS exposure were airborne nicotine and 3-ethenylpyridine (3-EP), and salivary cotinine. For purposes of assessing relative ETS exposure, the women were categorized according to husband's smoking status (*e.g.*, ETS-exposed if husband smokes). The relative exposures measured were: 8.6 for cotinine; 13.4 for nicotine; and 22.0 for 3-EP. These relative exposures are significantly larger than those assumed in recent ETS risk assessments and tend to question the validity of risk estimate corrections for so-called "background exposure." The same ratios measured for employed subjects indicate that workplace exposure is 10-fold lower than home exposure due to living with a smoker. In addition, these data also suggest that cotinine alone may be an inadequate marker for estimating ETS exposure in the general population. Median concentrations (all women) were found to be: 0.15 $\mu g/m^3$ (3-EP); 0.43 $\mu g/m^3$ (nicotine); and 0.8 ng/mL (salivary cotinine).

INTRODUCTION

An important issue in ETS risk assessment is that of background correction. As far as possible, epidemiologic studies employ control groups which are not exposed to the agent or action under investigation. Although tobacco use in the United States and the world is prevalent, and the potential exists for widespread exposure to ETS, some have claimed that ETS is ubiquitous (1,2) and make adjustments to risk estimates for exposure in control groups. Little is known about the magnitude of relative exposure in the population at large, and an upward adjustment to any risk estimate seems premature in light of the available evidence. In a recent risk assessment (2), the U.S. EPA adjusted the pooled U.S. risk estimate for a background correction factor (relative exposure) of 1.75. This factor is derived solely from relatively few measurements of cotinine. The generalizability to the U.S. population of much of these data is dubious, owing to the fact that virtually all of the studies cited were either of non-U.S. subjects or of a highly specialized subset of U.S. subjects (*e.g.*, only Hispanics, clinic patients and professional staff, etc.). Added to this is the observation that indices of exposure to ETS based on biologic markers, air monitoring, and questionnaires are not tightly correlated, and further, that single measures of ETS components or biologic markers are inadequate for characterizing normal exposure (3). In an attempt to partially rectify this lack of relevant data, the current study was designed. As such, this study has the unique distinction of;

(1) being representative of the U.S. female population with subject's ETS exposure status categorized exactly in accordance with the relevant epidemiology, (2) employing multiple markers of ETS exposure, and (3) measuring each marker over an extended time period.

SUBJECTS AND METHODS

Subject Selection and Study Design. Self-reported nonsmoking, married females (n=105) were recruited at random by a market research agency in Columbus, Ohio. Columbus was chosen as the test site due to its status as "one of the most average cities in the country" with its residents comprising a "demographically normal" segment of the U.S. population (4). A "nonsmoker" was defined as one who did not currently smoke and had not done so for at least six months prior to the study. A "never-smoker" was defined as one who had not ever smoked on a regular basis. Subjects were selected to fill cells defined by combinations of age, income, employment status, and husband's smoking status. The primary criteria were subject's employment status and husband's smoking status, for which cells were equally sized. For practical considerations, cells based on the remaining secondary criteria were only approximately equally sized. Subjects were considered employed if they worked full-time (35+ hours/week) outside the home and unemployed if they did not work at all outside the home. None of the subjects worked in a location with a smoking ban. Subjects were monitored continuously for ETS exposure (airborne nicotine and 3-ethenylpyridine) and nicotine intake (salivary cotinine) during one week (February, 1991) as they went about their normal daily routines. In addition, subjects married to smokers monitored airborne 3-EP and nicotine levels in their homes continuously for the week. Both pre- and post-sampling questionnaires were administered by trained interviewers at the agency's facility. Extensive data were gathered regarding dietary and lifestyle habits, subject whereabouts, and exposure to ETS. In addition, each subject maintained a daily activity diary during the study recording the same information on an hourly basis (excluding diet). Only the objective estimates of ETS exposure (nicotine, 3-ethenylpyridine, and cotinine), along with questionnaire responses regarding subject smoking history, are reported here.

Nicotine and 3-Ethenylpyridine Determination. All subjects wore a personal diffusion-based monitor continuously for five days except during sleep, when monitors were placed at bedside. In addition, wives of smoking husbands placed two monitors at fixed locations in their homes; one in a "heavy use" area and one in a "light use" area. Heavy and light use areas were targeted to be rooms where the husband did most of his in-home smoking and little, if any, smoking, respectively. Locations typically used by the participants were a den or living room for the heavy use area and an unused bedroom or a child's bedroom for the light use area. The monitors collected nicotine and 3-EP (a nicotine combustion product) on a sodium bisulfate-treated filter which was then extracted and analyzed by gas chromatography with *N*-thermionic detection (5). With an exposure duration of five days, limits of detection (LOD) for 3-EP and nicotine were 0.04 and 0.13 $\mu g/m^3$, respectively.

Cotinine Determination. Saliva samples were obtained by having the subject vigorously chew a Salivette (Sarstedt, Inc., Newton, NC) for 1 min. Four samples were obtained per subject, approximately one every other day, over a period of seven days (the first saliva sample was taken one to two days prior to the start of air monitoring). Salivettes were frozen immediately after sampling, transferred to the laboratory under dry ice, and stored frozen until analyzed. Duplicate analyses for cotinine were performed on each sample by

the radioimmunoassay method of Langoi
10 ng/mL was used to differentiate smol
of saliva in buffer, LOD for cotinine in

RESULTS

Of the 105 subjects recruited for this stu
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subject's workplaces. For employed su
them while at work.

Table 1. Analytical results (medians) o
husband's smoking status and subject's

Subject's husband smokes?	Subject employed?
yes	yes
no	yes
yes	no
no	no
yes	all subjects
no	all subjects
all subjects	yes
all subjects	no
all subjects	all subjects

[a]Median=0; value reported is LOD/2
[b]Median < LOD

Similarly, estimates of home ETS expo
Table 2. Data are presented according
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,er a period of seven days (the first saliva
start of air monitoring). Salivettes were
to the laboratory under dry ice, and stored
:otinine were performed on each sample by

the radioimmunoassay method of Langone (6). In general, a cut-point concentration of 10 ng/mL was used to differentiate smokers from nonsmokers (7). Using a 1:10 dilution of saliva in buffer, LOD for cotinine in saliva was determined to be 0.18 ng/mL.

RESULTS

Of the 105 subjects recruited for this study, 104 (70 reported never-smokers and 34 reported ex-smokers) successfully completed the sampling phase. Eight of these subjects (two reported never-smokers and six reported ex-smokers) were deemed to be active smokers ("deceivers") based on mean salivary cotinine values > 10 ng/mL, and their data were excluded from analysis. All four samples for each of these subjects contained cotinine in concentrations > 10 ng/mL. Three additional subjects, with borderline cotinine results, were retained. It is likely that these three subjects were occasional smokers; however, their data were retained nonetheless.

Misclassifying two out of 70 reported never-smoking subjects results in a never-smoker misclassification rate of 2.9%; misclassifying six out of 34 reported ex-smokers results in an ex-smoker misclassification rate of 17.6%; and, misclassifying eight out of 104 reported nonsmokers results in a nonsmoker misclassification rate of 7.7%.

Following deletion of the eight deceivers, summary statistics were computed for the objective estimates of personal ETS exposure in the remaining subjects, and the results are presented in Table 1. Data are presented according to all combinations of husband's smoking status and subject's employment status. Smoking was permitted in all of the subject's workplaces. For employed subjects, 37 of 45 (82%) observed smoking near them while at work.

Table 1. Analytical results (medians) of week-long personal monitoring according to husband's smoking status and subject's employment status.

Subject's husband smokes?	Subject employed?	n	3-EP ($\mu g/m^3$)	Nicotine ($\mu g/m^3$)	Cotinine (ng/mL)
yes	yes	23	0.66	2.17	2.37
no	yes	22	0.06	0.21	0.40
yes	no	25	0.36	0.87	2.125
no	no	26	0.02[a]	0.08[b]	0.23
yes	all subjects	48	0.44	1.61	2.33
no	all subjects	48	0.02[a]	0.12	0.27
all subjects	yes	45	0.19	0.89	0.93
all subjects	no	51	0.11	0.26	0.64
all subjects	all subjects	96	0.15	0.43	0.82

[a]Median=0; value reported is LOD/2
[b]Median < LOD

Similarly, estimates of home ETS exposure in the smoking households are summarized in Table 2. Data are presented according to all combinations of sampler location within the home and the subject's employment status. Relative exposure estimates were calculated as the ratio of median values in all pertinent categories in Tables 1 and 2, and the results are

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Table 2. Analytical results (medians) of week-long area monitoring in smoker's homes.

Home area*	Subject employed?	n	3-EP ($\mu g/m^3$)	Nicotine ($\mu g/m^3$)
light use	yes	23	0.68	1.14
light use	no	25	0.25	0.25
heavy use	yes	23	0.80	3.30
heavy use	no	25	0.62	1.50
avg light & heavy use	yes	23	0.81	2.39
avg light & heavy use	no	25	0.43	0.93
light use	all subjects	48	0.36	0.56
heavy use	all subjects	48	0.63	2.45
avg light & heavy use	all subjects	48	0.48	1.63

*For definitions of light and heavy use, see text.

summarized in Table 3. The ratios shown in row 1 (ratio between "smoking husband" and "nonsmoking husband" categories) employ the definition of exposed and unexposed subjects used in most epidemiology studies and conform to the definitions used by NRC (1) and EPA (2).

Table 3. Relative exposure estimates.

Relative exposure	3-EP	Nicotine	Cotinine
all subjects: S/NS husband	22.0††	13.4††	8.6††
unemployed subjects: S/NS husband	18.0††	10.9††	9.2††
employed subjects: S/NS husband	11.0††	10.3††	5.9††
all subjects: employed/unemployed	1.7*	3.4†	1.5*
subjects w/ NS husbands: employed/unemployed	3.0*	2.6†	1.7*
subjects w/ S husbands: employed/unemployed	1.8*	2.5†	1.1*
heavy home monitors: employed/unemployed	1.3*	2.2*	---
light home monitors: employed/unemployed	2.7†	4.6†	---
avg light & heavy: employed/unemployed	1.9*	2.6†	---
home monitors: heavy/light use areas	1.8*	4.4††	---

*Individual measures of central tendency not significantly different ($p > 0.05$)
†Individual measures of central tendency significantly different ($0.01 \leq p \leq 0.05$)
††Individual measures of central tendency significantly different ($p \leq 0.0003$)

DISCUSSION

Cotinine, a major nicotine metabolite with a half-life in humans of *ca.* 20 h (8), is considered the only viable biochemical marker of ETS exposure, although its use is not without limitations. Body burden of cotinine is presumed to reflect exposure to nicotine; however, exposure to nicotine does not necessarily equate with exposure to ETS. Due to unusual decay characteristics, nicotine lingers in many environments long after other

tobacco smoke constituents have disap
overestimating ETS exposure (9). Th
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Ethenylpyridine, a combustion produc
exposure since 3-EP decays at rates si
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The concentrations of the airborne ma
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virtually all of their ETS exposure co
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·g area monitoring in smoker's homes.

n	3-EP ($\mu g/m^3$)	Nicotine ($\mu g/m^3$)
23	0.68	1.14
25	0.25	0.25
23	0.80	3.30
25	0.62	1.50
23	0.81	2.39
25	0.43	0.93
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48	0.48	1.63

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	3-EP	Nicotine	Cotinine
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	18.0††	10.9††	9.2††
	11.0††	10.3††	5.9††
	11.7*	3.4†	1.5*
:d	3.0*	2.6†	1.7*
	1.8*	2.5†	1.1*
	1.3*	2.2*	---
	2.7†	4.6†	---
	1.9*	2.6†	---
	1.8*	4.4††	---

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e in humans of *ca.* 20 h (8), is
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ny environments long after other

tobacco smoke constituents have disappeared, resulting, in many instances, in nicotine overestimating ETS exposure (9). This, along with possible consumption of nicotine in the diet and large differences in individual rates of metabolic clearance (10), limits the quantitative application of nicotine and cotinine in exposure assessment at low doses. 3-Ethenylpyridine, a combustion product of nicotine, is a more objective estimate of ETS exposure since 3-EP decays at rates similar to other ETS components (including particles) (11,12).

The concentrations of the airborne markers (Table 1) indicate that long-term personal exposures are considerably lower than predicted from short-term area monitoring data (13), even for the most heavily exposed group (*i.e.*, nonsmokers married to smokers). This is due primarily to the inclusion of significant portions of sampling time in the absence of ETS (during sleep, travel, etc.) and is, of course, a more accurate reflection of true exposure. From a population perspective, it appears that for wives of smokers, virtually all of their ETS exposure comes from the home. The 3-EP and nicotine median concentrations from personal monitoring (Table 1, rows 1 and 3) are essentially equivalent to the median average home concentrations (derived from averaging the light and heavy monitor data from each home; Table 2, rows 5 and 6).

The relative exposure data in Table 3 support several important conclusions. First, the magnitude of exposure ratios between the "smoking husband" and the "nonsmoking husband" category (Table 3, row 1) suggests that factors used in recent risk assessments (*e.g.*, background correction factor "Z" = 1.75 (2)) are in error for the U.S. population. The data reported here from three markers measured over a fairly long time period in a demographically representative cross-section of U.S. women suggest true values of "Z" are at least five- to ten-times greater. Second, the same ratios measured for employed subjects (Table 3, row 3) indicate that workplace exposure due to working with smokers is approximately 10-fold lower than home exposure due to living with a smoker. It is presumed by many that the only major source of ETS exposure for a nonsmoker not living with a smoker is the workplace (2,14,15). While this may be true, the extent of workplace exposure appears to be an order of magnitude lower than "spousal smoking" exposure. Such data contradict recent opinion that "[w]orkplace ETS levels are generally comparable with home ETS levels, and studies using body cotinine measures as biomarkers demonstrate that nonspousal exposures to ETS are often greater than exposure from spousal smoking" (2, p. 1-12). A third important conclusion from the data reported here is that interpretation of workplace exposure based upon nondiscriminating data (*e.g.*, questionnaire data, 24-hour exposure monitoring, cotinine measures, etc.) may lead to spurious conclusions. Such measures cannot discriminate among home, workplace and other exposures in the way that discreet air monitoring in specific locations can. For instance, Table 3 (row 6) indicates a 2.5-fold increase in the week-long nicotine monitor data for employed wives of smokers compared with unemployed wives of smokers (the same trend is noted for 3-EP monitor data; however, the difference is not statistically significant). A cursory analysis might conclude that employed wives of smokers have significant additional exposure from the workplace over their unemployed counterparts. We note that just such a conclusion was reached recently (15) based on questionnaire data. However, analysis of the relative magnitude of home exposure between these two categories (Table 3, rows 7-9) demonstrates that home exposures are two-fold higher for employed wives of smokers. Consequently, the apparent workplace effect is misleading and is, instead, a confounded measure of home exposure. There is no difference in the observed in-home smoking behavior of husbands between the two categories; however,

homes in the employed subject category were significantly smaller than homes in the unemployed subject category. Thus, we conclude that the increased home exposure is due to the smaller home size; a potential socioeconomic confounder. When the week-long personal monitoring data are corrected for exposure in the home (reported time at home combined with average home monitor data), the apparent workplace effect disappears. Also of interest in this same category are the virtually identical median levels of salivary cotinine (compared to a significant 2.5-fold difference in nicotine exposure and an insignificant 1.8-fold difference in 3-EP exposure). This suggests that of the three markers used in this study to characterize ETS exposure, cotinine is the least discriminating. In conjunction with additional problems outlined previously, and the noted overall trend in differentiating ability (Table 3, row 1) among the markers, cotinine appears to significantly underestimate true differences in ETS exposure.

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[6]British Gas (West Midlands), Solihul

ABSTRACT

A longitudinal cohort study was cond
Index (BSI) is attributable to exposure
smokers.

A cross-sectional analysis of the initia
smoking status could be validated thro
BSI and perceived ETS exposure were
levels adjacent to smokers were signif
found between the Personal Symptom

It is concluded that smoking status an
on the BSI than objectively measured

INTRODUCTION

In a study of 4,373 office workers, Ro
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Environmental Tobacco Smoke (ETS)
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Determination of Volatile Organic Compounds and ETS Apportionment in 49 Homes

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Forty-nine nonsmoking married women participated in a home personal exposure study for 28 target volatile organic compounds (VOCs) and total volatile organic compounds (TVOCs). The women were selected and classified according to 18 socioeconomic categories based on age (18-34, 35-49, 50-64), family income (<$25K, $25K-$40K, >$40K), and husband's smoking status. Of the 29 analytes, 21 demonstrate no statistically significant difference in exposure between nonsmoking and smoking homes. One VOC, trichloroethylene, is elevated in the nonsmoking homes and seven VOCs, benzene, styrene, pyridine, 2-picoline, 3-picoline, 3-ethylpyridine, and 3-ethenylpyridine, are elevated in the smoking homes. A correlation matrix and a factor analysis indicate that benzene and styrene are not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor phase environmental tobacco smoke (ETS) marker. All of the nitrogenous bases are significantly correlated with 3-ethenylpyridine. Benzene, styrene, and TVOC are not significantly correlated with the number of cigarettes smoked; however, 3-ethenylpyridine is significantly correlated with the number of cigarettes smoked. A Pearson correlation analysis indicates that "gas heat" and "smoking husband" are significantly correlated with elevated benzene concentrations, but a multiple regression model for benzene accounts for less than 30% of the total variance. ETS variables account for only 8% of the total variance. In the smoking homes, an apportionment technique is evaluated for selected VOCs in order to determine the median percentage of each analyte attributable to ETS. The results, with percentages

attributable to ETS are: TVOC (5.5%), benzene (13.2%), styrene (12.6%), pyridine (40.7%), 2-picoline (67.1%), 3-picoline (90.1%), 4-picoline (37.2%), and 3-ethylpyridine (62.0%). Indoor air sources other than ETS are also identified for limonene, tetrachloroethylene, 1,4-dichlorobenzene, and alkylbenzenes.

Introduction

Studies on human exposure to target volatile organic compounds (VOCs) have increased dramatically in recent years. A few of the studies concentrate on outdoor aliphatic and aromatic VOC exposure while driving automobiles (Adlkofer et al. 1990; Chan et al. 1991; Dasch and Williams 1991), riding bicycles on urban and suburban thoroughfares (Bevan et al. 1991), or walking on sidewalks near busy city streets (Chan et al. 1991), but most of the studies focus on measurements in indoor air environments (Adlkofer et al. 1990; Bayer and Black 1986; Bayer and Black 1987; Brown et al. 1990; Chan et al. 1990; Girman et al. 1986; Higgins et al. 1987; McKone and Knezovich 1991; Michael et al. 1990; Miksch et al. 1982; Mølhave 1982; Mølhave 1990; Proctor 1988; Proctor 1989; Proctor et al. 1989; Proctor et al. 1991; Wallace 1986a; Wallace 1986b; Wallace 1987; Wallace et al. 1987; Weschler et al. 1990). Indoor VOC concentrations are generally higher than outdoor concentrations, and since people tend to spend most of their time indoors at home or in the workplace, the potential for VOC exposure is greatest in indoor settings (Wallace 1987a). Although simple quantitation of target VOCs is meaningful, source characterization and apportionment are vital and integral

parts of any indoor air evaluation. Numerous sources of indoor air VOCs exist: unvented kerosene or oil heaters, gas or oil furnaces, gas stoves, consumer products such as air fresheners, moth balls, and toilet bowl deodorizers, building or furnishing materials, clothing, use of hot water, and infiltration of VOCs from outdoor sources. However, one of the most controversial and publicized sources of indoor air VOCs is environmental tobacco smoke (ETS). The VOCs contributed by ETS constitute a heretofore undetermined fraction of a complex, multisource mixture of indoor air volatile compounds.

Tobacco does contribute to VOC levels in indoor environments by the very nature of cigarette use; that is, sidestream smoke and exhaled mainstream smoke generated from cigarettes as a result of combustion or pyrolysis mechanisms consequently emit VOCs into the surrounding environment. In a recent study (Heavner et al. 1992), a technique of ETS apportionment was proposed to assess the effect of smoking activity on specific VOC concentrations. The technique is based on the ratio of 3-ethenylpyridine, a vapor phase ETS marker, to other VOCs found in ETS.

The purpose of this investigation is to conduct a personal VOC exposure study of "smoking" and "nonsmoking" homes in a demographically selected U.S. city without the confounding effect of workplace exposure and to (1) determine the concentration of TVOCs (Wallace et al. 1991) and 28 target VOCs in these homes; (2) compare the VOC and TVOC levels in smoking and nonsmoking homes; (3) develop a correlation matrix to

compare VOC and TVOC concentrations with respect to each other; (4) assess the effect of specific lifestyle activities on selected VOC concentrations; (5) evaluate the relationship between the proposed vapor phase ETS marker, 3-ethenylpyridine, and other indicators of smoking activity such as indoor air nicotine and salivary cotinine concentrations; and, most importantly, (6) evaluate the ETS apportionment technique in order to determine the contribution of ETS to TVOC and selected target VOC concentrations in indoor air environments.

Experimental Methods

Apparatus, Materials and Chemicals. Personal VOC multisorbent samplers consisted of two stainless steel cartridges connected in series and packed with 160 mg of 60/80 mesh Tenax TA (Alltech Associates, Deerfield, IL) and 160 mg of 60/80 mesh Carbotrap (Supelco Inc., Bellefonte, PA) in each cartridge. The solid adsorbent sample cartridges were connected to Model 222-4 "low-flow" diaphragm pumps (SKC South, Appomattox, VA) with approximately 30 cm of silicone rubber tubing. A pen clip was connected onto the front cartridge to facilitate attachment of the sampler cartridges to a lapel, collar, or pocket in the breathing zone of the participant. Pump flows (60-80 mL/min) were measured before and after the sample period, and the average of the two flows was used to calculate the VOC concentrations. Sample cartridges were analyzed by thermal desorption/gas chromatography/mass spectrometry for TVOCs and 28 target VOCs. Collection efficiency correction factors for each VOC were calculated and applied in

order to determine the amount entering the front cartridge while [illegible] were obtained by summing the mass determined on the front and back cartridges. In addition, ten cartridges were used for field blank correction. Data were analyzed statistically with SAS® software (SAS Institute Inc., Cary, NC). Sampler construction, sample collection, and analytical techniques are described in detail elsewhere (Heavner et al. 1992).

Diffusion monitors or passive sampling devices (PSDs) for five-day time-weighted average nicotine and 3-ethenylpyridine determinations were constructed from three-piece 37-mm polystyrene filter cassettes (Millipore Corp., Bedford, MA) with a Teflon membrane filter windscreen (Schleicher & Schuell, Keene, NH) and a glass fiber filter (Pallflex Co., Putnam, CT) treated with a 4% sodium bisulfate (Aldrich Chemical Co., St. Louis, MO) solution as the collection medium. Prior to, and immediately following sample collection, the PSDs were stored in polypropylene specimen jars with screw-cap lids (Fisher Scientific, Pittsburgh, PA). After sample preparation, filters were analyzed by gas chromatography/thermionic-specific (nitrogen) detection. PSD sampling rates for nicotine and 3-ethenylpyridine were 31.5 mL/min and 27.8 mL/min, respectively. PSD construction, validation, determination of sampling rates, sample preparation, and analytical techniques are described in detail elsewhere (Ogden and Maiolo 1992; Ogden et al. 1993).

Saliva samples were collected with Salivettes (Sarstedt Inc., Newton, NC). The devices

consisted of clear polystyrene conical centrifuge tubes with a snap-on [illegible] untreated cylindrical cotton swab. After saliva collection, the Salivettes were placed in individual envelopes and stored at approximately -10 °C in a freezer prior to cotinine determination by radioimmunoassay (RIA). The RIA method is described in detail elsewhere (Langone 1973; Langone and Van Vunakis 1982).

All sample media (VOC samplers, PSDs, and Salivettes) were collected from the participants over a five-day period and stored at approximately -10 °C in freezers at a central location. After all sample collection materials were received, the media were packed with dry ice and shipped to the laboratory where they were immediately transferred to freezers and stored until analysis.

Site Selection and Timing. A national market research agency was contracted to provide guidance and assistance with regard to selection of a site city and study dates, development of a questionnaire and diary, participant training, and general administrative aspects of the VOC project. A local field service in the site city was subcontracted to provide a central facility for participant recruitment/training and collection of sample materials. Also, the national market research agency provided expertise as a liaison between the local field service, study participants, and client technical personnel involved in the study to ensure that neither the participants nor the local field service personnel were aware of the purpose of the study or the company sponsoring the project.

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Columbus, Ohio during the winter week of February 25-29, 1991 was selected as a suitable site and time. Columbus is described by the American Automobile Association (AAA) as "one of the most average cities in the country" because its residents comprise a "demographically normal" segment of the U.S. population (Illinois/Indiana/Ohio Tourbook 1991). Also, the AAA states that Columbus offers a "perfect cross section of consumers" and is often referred to as "Test Market, U.S.A." Columbus has a population of approximately 560,000 with a metropolitan area population of over one million residents. In addition, Columbus is known as a center for scientific and technical information, retail banking, insurance, and real estate without the traditional heavy industrial base found in many midwestern U.S. cities.

Questionnaire and Diary Development. Questionnaires and diaries were developed in a joint effort with the national market research agency, client researchers, and a client statistician to ensure that questions were concise and unambiguous for participants in all socioeconomic categories. Questions related to lifestyle, leisure activities, diet, etc. were included and arranged to conceal the fact that the major independent variable was the husband's smoking status. The following questionnaires, diaries, and instructional materials were developed as a result of these efforts: (1) a "Screening Questionnaire" form used for initial telephone recruitment of participants; (2) a "First Visit Survey" form used by participants upon entering the site market research agency facility; (3) a "Pump and Special Diary Instructions" form used by participants as instructional reference for VOC sampler/pump operation and diary completion; (4) a "Special Diary and Additional

Questions" form used to record activities during the VOC sample period; (5) an "Air Monitor: Instructions for Participant" form used to instruct and record information regarding the use of personal and fixed area PSDs in the homes; (6) a "Saliva Sampling" form used to instruct and record information regarding collection of saliva samples; (7) a "Monitor/Salivette Return Probe" form used by interviewers to question participants regarding placement, usage, and collection of PSDs and saliva samples; (8) a "Pump Return Probe" form used to question participants regarding operation of the VOC sampler/pump; and (9) a "Last Visit Survey" form used to question participants regarding hobbies, diet, use of consumer products, etc.

Each prospective married female participant was placed in one of 18 cell categories based on the following variables: the husband's smoking status (smoking or nonsmoking), the participant's age (18-34, 35-49, or 50-64 years old), and family income range (<$25K, $25-$40K, or >$40K per year). Homes that contained smoking household members other than the husband were rejected. Fifty-five participants were recruited by telephone from the local field service's in-house database for participation in the project representing 24 of the 57 residential area Zip Codes in the greater Columbus area. All 18 cell categories were filled with at least one participant in each cell.

Participant Training. Experienced interviewers from the local field service trained all female participants. Each participant was given two kits: a VOC kit and a PSD/saliva kit. The VOC kit contained instructional materials, an activity diary, a pre-calibrated

[illegible] sampler/pump, and a small apron in which to place the pump during the sampling period. The instruction form included a diagram of the sampler/pump and information regarding pump operation. The interviewer demonstrated the proper technique for wearing the VOC sampler with the pump in the apron and the multisorbent sampler cartridges clipped onto a lapel or collar. The procedures for turning the pump on and off, recording the start and stop times, and completing the "Special Diary and Additional Questions" forms were conveyed as well.

VOC Sampling. The participants wore the VOC sampler for three consecutive evening hours on either Monday, Tuesday, or Wednesday during the week of February 25-29, 1991 while both the husband and wife were at home. In addition, the participants completed a special activity diary segmented into 15-minute intervals over the three-hour sampling period. The female participant recorded activities pertaining to everyone in the household. On the day after collecting the three-hour VOC sample, the participants returned the sampler to the facility. The recorded pump start and stop times were verified, and the pump flow was measured for comparison to the initial pump calibration. Finally, the participants signed a form at the end of the study certifying that they operated the pump for three consecutive hours and recorded activities that occurred during that period.

Nicotine and 3-Ethenylpyridine PSD Sampling. All participants wore a personal PSD for five consecutive days. In the smokers' homes only, a PSD was placed for five

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consecutive days in a "light" use area of the home where [illegible] least amount of time together, e.g. a child's bedroom, and a "heavy" use area [illegible] where the husband and wife spent most of their time together, e.g. a family room or den. The participants were told that the PSD would detect odors and chemicals; the participants were not told that the PSD was specific for nicotine or 3-ethenylpyridine. Daily diaries were provided for each of the five days, Monday through Friday, February 25-29, 1991. Daily diaries were segmented into one-hour periods for recording activities that occurred. During that hour, the participants were asked to record the number of cigarettes that were smoked in their presence, the number of times they smelled exhaust fumes, and the number of times that other odors were noticed. Each participant was given the appropriate number of PSDs, capped and placed in screw-cap jars, to open on the morning of the first day of the study (Monday). On Friday, the participants were asked to place the PSDs in the jars, record the time at the end of the study, and return the materials to the market research facility. Upon returning to the facility with the PSDs and saliva samples on the last day of the study, the participants were questioned regarding use of the sample materials.

Saliva Sampling. Also included in the PSD/saliva kit were the saliva sample instructional materials, four Salivette tubes, a thermally insulated bag, a refreezable ice pack (Stanbel Ice-Pak Inc., Montreal, PQ), and four insulated envelopes. To illustrate proper use of the saliva collection system during the first visit, the participants were asked to remove one of the Salivettes, place the cotton swab in their mouth, and chew

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vigorously for one minute. The cotton swan was returned to the tube, and the tube was inserted into one of the envelopes. The envelopes were collected and immediately placed in a freezer for a baseline cotinine determination. These samples were labelled as "Demo" for administrative purposes. The participants were instructed to collect similar saliva samples on Monday morning, Wednesday evening, and Friday evening. The participants were instructed to place all saliva samples in individual envelopes and to store the envelopes in a freezer immediately. On Friday, February 29, the participants were asked to place the frozen ice pack in the thermal bag with the three saliva samples and return the bag to the market research facility.

Results and Discussion

Participant Rejection Criteria. Fifty-five married females participated in the sample collection portion of the Columbus study: 27 married to nonsmokers and 28 married to smokers. However, based on salivary cotinine results greater than 10ng/mL, five women were eliminated from the data analysis portion of the study (Etzel 1990). Another woman married to a smoker was eliminated from the data analysis portion of the study when it was discovered that the room in which she and her husband spent the majority of their time was equipped with an air cleaning system and that her husband smoked in a separate room of the house. Inclusion of this participant's results in the smoking home category would potentially bias the results. As a result, 49 participants were included in the data analysis portion of the study: 24 women married to nonsmokers and 25 women

married to smokers.

VOC Summary Statistics. Summary statistics for all VOCs and TVOCs are reported in Table I. Results are separated into smoking and nonsmoking homes with the number of determinations for each analyte (n), the means, standard deviations, medians, minima, maxima, and Wilcoxon Rank Sum Test p values for determining statistically significant differences between smoking and nonsmoking homes. The Wilcoxon Rank Sum Test was used because the data are generally not normally distributed. Of the 29 analytes (TVOC and 28 VOCs), 21 demonstrate no significant difference between smoking and nonsmoking homes. One of the VOCs, trichloroethylene, is elevated in the nonsmoking homes. Seven of the VOCs are elevated in the smoking homes: benzene, styrene, pyridine, 2-picoline, 3-picoline, 3-ethylpyridine, and 3-ethenylpyridine (3-EP). Of these seven, four of the nitrogenous bases (pyridine, 2-picoline, 3-picoline, and 3-ethylpyridine) are generally considered to originate predominantly from tobacco sources, and, as a result, are elevated in the smoking homes. However, pyridine and many of the pyridine derivatives have been found at trace levels in foods such as fish, meat, vegetables, cereals, dairy products, and alcoholic beverages (Jori et al. 1983; Vernin 1982). In addition, pyridine is eliminated from the human body through the breath, urine, feces, and skin (Jori et al. 1983), and a number of the pyridine derivatives are used as chemical intermediates in the production of adhesives, fibers, resins, and pesticides (Windholz 1976). Therefore, detection of some of these nitrogenous bases is expected in homes without smoking activity. The other nitrogenous base, 3-EP, is considered tobacco-

specific and is a proposed vapor phase ETS marker (Eatough et al. 1989; Heavner et al. 1992). Of course, 3-EP is elevated in the smoking homes, thus providing a measure of smoking activity. However, 3-EP is found in four of the 24 nonsmoking homes, though at extremely low concentrations. The mean 3-EP concentration in nonsmoking homes is 0.08 $\mu g/m^3$, while the mean 3-EP concentration in smoking homes is 1.28 $\mu g/m^3$; corresponding median concentrations are less than the limit of detection (LOD) and 0.95 $\mu g/m^3$, respectively. The maximum 3-EP concentration in the nonsmoking homes is 0.57 $\mu g/m^3$, while the maximum 3-EP concentration in the smoking homes is 5.58 $\mu g/m^3$.

Correlation Matrix. A matrix of Pearson correlation coefficients is presented in Table II to illustrate statistically significant correlations between individual VOCs and, ultimately, VOC compound classes; correlation results from all homes (n=49), smoking and nonsmoking, are included in the matrix. Significant correlations at $p \leq 0.05$ are represented by filled circles (●) while correlations at $0.05 < p \leq 0.10$ are represented by open squares (□). The proposed ETS marker, 3-EP, is significantly correlated at $p \leq 0.05$ with the five nitrogenous bases: pyridine (r=0.65), 2-picoline (r=0.85), 3-picoline (r=0.88), 4-picoline (r=0.55), and 3-ethylpyridine (r=0.64). Also, limonene (r=0.24) is correlated at $0.05 < p \leq 0.10$ with the ETS marker. Although benzene is elevated in smoking homes, benzene is not correlated with 3-EP at either significance level. However, benzene is significantly correlated with 2-picoline (r=0.34) at $p \leq 0.05$ and 3-ethylpyridine (r=0.26) at $0.05 < p \leq 0.10$. In the smoking homes (n=25), benzene is not significantly correlated with either 3-EP or any of the nitrogenous bases. Thus, the

[illegible] correlation between benzene and "tobacco-related" nitrogenous bases in all [illegible] smoking homes is insignificant. Instead, in all houses, benzene is significantly correlated with a number of other aromatic VOCs: toluene ($r=0.41$), ethylbenzene ($r=0.46$), *o*-xylene ($r=0.45$), *m*-xylene ($r=0.49$), *p*-xylene ($r=0.44$), *n*-propylbenzene ($r=0.36$), *n*-butylbenzene ($r=0.30$), 1,2,3-trimethylbenzene ($r=0.28$), and 1,3,5-trimethylbenzene ($r=0.35$) at $p \leq 0.05$, and styrene ($r=0.26$) at $0.05 < p \leq 0.10$. Correlation coefficients of styrene with other VOCs are similar to those of benzene. Styrene is correlated at $p \leq 0.05$ with ethylbenzene ($r=0.35$), *o*-xylene ($r=0.36$), *m*-xylene ($r=0.34$), *p*-xylene ($r=0.35$), pyridine ($r=0.30$), *n*-propylbenzene ($r=0.28$), 2-picoline ($r=0.36$), 3-picoline ($r=0.28$), 1,2,3-trimethylbenzene ($r=0.31$), and 1,3,5-trimethylbenzene ($r=0.29$). Styrene is not significantly correlated with 3-EP, the vapor phase ETS marker. As is the case with benzene, styrene in the smoking homes ($n=25$) is not significantly correlated with either 3-EP or any of the "tobacco-related" nitrogenous bases at $p \leq 0.05$ or $0.05 < p \leq 0.10$. In general, correlation results presented in Table II demonstrate that aromatic hydrocarbons are correlated with aromatic hydrocarbons, aliphatic hydrocarbons are correlated with aliphatic hydrocarbons, and nitrogenous bases are correlated with nitrogenous bases.

Factor Analysis. Since a visual inspection of the correlation matrix suggests that the VOCs might occur in correlated groups, a factor analysis was performed on all 28 VOCs and TVOC. Factor analysis is a variable-directed statistical technique used to identify or derive new variables or factors that provide a better understanding of the data (Chatfield

and Collins 1980). The purpose of performing a factor analysis is to examine the covariance structure of variables and to identify factors that are related to groups of variables. The interpretation of factors is a subjective process, generally aided by rotating the factors so that each factor is either highly correlated with a given variable or uncorrelated with it. Then the factor can be said to be representative of commonalities in the set of variables with which it is highly correlated and free of association from the set of variables with which it is not correlated. Since a number of major indoor air VOC sources are known to exist, it is reasonable to expect that many of the VOCs are generated from a smaller number of unique, unrelated sources that can be isolated as groups of interrelated variables in a factor analysis.

Factor analysis results for the 29 analytes are reported in Table III. Seven factors are identified with the variance explained by each factor listed above the VOCs grouped in that factor. The variance explained is closely related to the number of variables accounted for by each factor group. VOCs with a correlation coefficient of $r \geq 0.50$ with each factor are listed in Table III to show how the factor analysis grouped the VOCs. Factor one contains only aromatic hydrocarbons. Factor two contains mostly aliphatic hydrocarbons and TVOC with an overlap of one aromatic hydrocarbon, isopropylbenzene. Factor three contains only the "tobacco-related" nitrogenous bases including 3-EP, the vapor phase ETS marker. Factor four contains 1,4-dichlorobenzene and two *n*-alkanes (*n*-nonane, *n*-decane) with which it is significantly correlated in the correlation matrix (Table II). Factor five contains tetrachloroethylene and toluene;

tetrachloroethylene is significantly correlated with toluene and no other VOC (Table II). Factor six contains trichloroethylene and limonene, and factor seven contains styrene. In general, the factor analysis demonstrates the presence of three main groups incorporating 22 of the 29 analytes. These groups are characterized by compound class: the aliphatic VOCs, the aromatic VOCs, and the "tobacco-related" nitrogenous bases. The remaining seven analytes are distributed among four smaller, miscellaneous groups. The most significant finding is that benzene is grouped with the aromatic VOCs and not the "tobacco-related" nitrogenous bases. In addition, styrene is in a factor grouping by itself and is not grouped with the "tobacco-related" nitrogenous bases. If ETS were the sole or even the predominant indoor air source of benzene or styrene, then these two compounds would be expected to group with the "tobacco-related" VOCs.

Miscellaneous VOC Source Identification. The VOC target list of 28 compounds includes chemicals that originate from a number of diverse sources. In order to establish sources of these compounds, questionnaire responses were reviewed and specific activities or consumer products were selected as independent variables to determine statistically significant differences in the dependent variable responses, *i.e.* selected VOC concentrations. No attempt was made to identify sources for all of the target VOCs. Instead, a select group of four compounds or classes of compounds was chosen for the miscellaneous source identification: limonene, tetrachloroethylene, 1,4-dichlorobenzene, and the aromatic hydrocarbons. ETS as a source of specific VOCs will be addressed later in this discussion.

Limonene is a naturally-occurring compound found in the essential oils of a number of citrus fruits and herbs such as lemons, oranges, caraway, dill, and bergamot (Windholz 1976). Because of its lemon-like odor, it is often used in the consumer product industry as a fragrance to improve the sensory acceptability of products. In the "Last Visit Survey," participants were asked if they used certain consumer products on a fairly regular basis. Twelve consumer products were selected as potential sources of limonene: air freshener, liquid bleach, furniture polish, hair spray, kitchen cleaners, window cleaner, fingernail polish, carpet freshener, carpet cleaner, stain remover, perfume, and bug killer. Exposure to ETS during the three-hour "pump period" was selected as an additional independent variable for testing limonene concentrations since limonene is correlated with 3-EP at $0.05 < p \leq 0.10$ (Table II). In Table IV, Wilcoxon Rank Sum Test results are presented to compare limonene levels in the households where potential source products were used to households where they were not used, with significance indicated at $p \leq 0.05$. Results are tabulated separately for smoking/nonsmoking homes and all homes combined. In the nonsmoking homes, elevated limonene concentrations are associated with the use of hair spray or perfume ($p \leq 0.05$), and air freshener, furniture polish, and stain remover ($0.05 < p \leq 0.10$). In the smoking homes, elevated limonene concentrations are associated with the use of stain remover ($p \leq 0.05$). In all homes combined, elevated limonene concentrations are associated with the use of furniture polish and stain remover ($p \leq 0.05$ level) and hair spray ($0.05 < p \leq 0.10$). Exposure to ETS during the pump period appears to be statistically unrelated to limonene concentrations in the smoking homes even though limonene is correlated with 3-EP in

Tetrachloroethylene is a known commercial dry-cleaning solvent, and is often used as a degreasing agent in commercial organic solvent-based cleaners (Windholz 1976). Trichloroethylene has been used in the past as a commercial dry-cleaning solvent; however, current applications include use as a degreasing agent and as a solvent for numerous chemical preparations and coatings, such as paints and varnishes (Juhola 1973; Windholz 1976). In Table V(a), Wilcoxon Rank Sum Test results are presented to compare levels of trichloroethylene and tetrachloroethylene for groups of households defined by the activity of wearing clothes that were dry-cleaned within the previous week. Results are tabulated separately for smoking and nonsmoking homes and all homes combined with significance indicated at $p \leq 0.05$. In the nonsmoking homes and smoking homes separately, neither trichloroethylene nor tetrachloroethylene concentrations are significantly elevated with the activity of wearing dry-cleaned clothes; however, when all homes are combined, elevated tetrachloroethylene concentrations are associated with the activity of wearing dry-cleaned clothes ($p \leq 0.05$). Statistical power for this test is low since only two of the 49 participants wore clothes that had been dry cleaned within the previous week.

1,4-Dichlorobenzene is a volatile, crystalline solid used as an insecticidal fumigant in domestic mothball preparations, air fresheners/deodorizers, and toilet bowl deodorizers (Wallace 1987a; Windholz 1976). In Table V(b), Wilcoxon Rank Sum Test results are

presented to compare levels of 1,4-dichlorobenzene for groups of households defined by the activity of using mothballs in the home. Results are tabulated separately for smoking and nonsmoking homes and all homes combined with significance indicated at $p \leq 0.05$. In the nonsmoking homes and smoking homes separately, 1,4-dichlorobenzene concentrations are not significantly correlated with the activity of using mothballs in the home; however, when all homes are combined, elevated 1,4-dichlorobenzene concentrations are associated with the activity of using mothballs ($p \leq 0.05$). The ability to detect a significant effect in all homes combined is apparently due to an increase in statistical power.

A typical commercial grade of gasoline is a mixture of C_4 to C_{12} hydrocarbons containing aromatics, paraffins, and olefins (Winholz 1976)). It is a volatile mixture that could potentially affect indoor VOC concentrations, if gasoline is stored in a basement or attached garage. In Table VI, Wilcoxon Rank Sum Test results are presented to compare levels of the aromatic hydrocarbons and households defined by the activity of storing gasoline in an attached garage or basement. Again, results are tabulated separately for the smoking and nonsmoking homes and all homes combined with significance indicated at $p \leq 0.05$. In the nonsmoking homes, *o*-xylene ($p \leq 0.05$), toluene, isopropylbenzene, and *n*-propylbenzene ($0.05 < p \leq 0.10$) concentrations are significantly elevated in homes where gasoline is stored. In the smoking homes, *p*-xylene, *o*-xylene, *n*-propylbenzene ($p \leq 0.05$), *m*-xylene, and 1,3,5-trimethylbenzene ($0.05 < p \leq 0.10$) concentrations are significantly elevated in homes where gasoline is stored. In all

homes combined, ethylbenzene, *p*-xylene, isopropylbenzene, *o*-xylene, *n*-propylbenzene, 1,3,5-trimethylbenzene, 1,2,3-trimethylbenzene ($p \leq 0.05$), and *m*-xylene ($0.05 < p \leq 0.10$) are significantly elevated in homes where gasoline is stored. It is somewhat surprising that benzene and toluene do not demonstrate significance at either level since benzene and toluene are known to exist in gasoline formulations and automobile exhaust (Robinson et al. 1988).

Three-Hour Versus Five-Day Smoking Activity. The three-hour VOC samples collected in each of the 49 homes provide no information related to activities and subsequent exposures that occur on a daily, weekly, or yearly basis. In essence, the VOC samples collected in the homes provide a three-hour "snapshot" of activities and VOC exposure; however, it is important to establish whether or not the three-hour sampling period is typical of, or at least related to, longer-term smoking activity and VOC exposure. In Table VII, results are presented for a Pearson correlation analysis comparing the three-hour 3-EP concentrations with the five-day PSD nicotine, the five-day PSD 3-EP, and the salivary cotinine concentrations. Significant correlations at $p \leq 0.05$ are demonstrated for all comparisons with the exception of the Monday morning salivary cotinine ($0.05 < p \leq 0.10$). In general, the correlation analysis results suggest that the three-hour VOC sampler 3-EP concentrations are typical of the week's smoking activity.

For 3-ethenylpyridine, the three-hour 3-EP concentrations are significantly correlated ($p \leq 0.05$) with the five-day 3-EP "light" PSD ($r=0.88$), the five-day 3-EP "heavy" PSD

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(r=0.88), and the five-day 3-EP average PSD (r=0.89) concentrations. Thus, a strong statistical relationship exists between the short-term (three hour) 3-EP and the long-term (five day) 3-EP concentrations. In contrast, although the three-hour 3-EP concentrations are significantly correlated with the five-day 3-EP personal PSD (r=0.72) concentrations, the relationship is somewhat weaker.

The three-hour VOC sampler 3-EP and the five-day PSD nicotine relationship is similar. For example, the three-hour 3-EP concentrations are significantly correlated ($p \leq 0.05$) with the five-day nicotine "light" PSD (r=0.46), the five-day nicotine "heavy" PSD (r=0.84), and the five-day nicotine average PSD (r=0.78) concentrations. Again, the direct comparison of short-term 3-EP with long-term nicotine demonstrates a strong, statistically significant relationship with the exception of the "light" monitor which has the smallest correlation coefficient. Given the decay properties of nicotine (Nelson et al. 1990) and the location of the "light" monitor in the child's bedroom or other seldom used area distant from the majority of smoking activity, a weaker correlation is expected.

For salivary cotinine, the three-hour 3-EP concentrations are significantly correlated ($p \leq 0.05$)with the "Demo" sample (r=0.61), the Wednesday evening sample (r=0.71), the Friday evening sample (r=0.64), the average of all samples (r=0.62), and the Monday morning salivary cotinine (r=0.36) at $0.05 < p \leq 0.10$. Since cotinine has a half-life of approximately 20 hours in humans (Benowitz 1983), all of the salivary cotinine samples, with the exception of the Monday morning sample, are indicative of exposure that

occurred during the week; the Monday morning sample is indicative of exposure that occurred on a weekend. If one assumes that weekday activities differ from weekend activities, then the Monday morning saliva sample is less typical of exposures that occur during the week. Overall, the three-hour VOC sampler 3-EP concentrations are more strongly correlated with the five-day PSD determinations, 3-EP and nicotine, than with salivary cotinine concentrations. Since the metabolic response of individuals to nicotine exposure is highly variable (Idle 1990), it is not surprising that the relationship between salivary cotinine concentrations and VOC sampler 3-EP concentrations is not as strong as the relationship between the PSD concentrations and VOC sampler 3-EP concentrations.

Number of Cigarettes Smoked. Information on the number of cigarettes observed during the "pump period" was recorded on the " Special Diary and Additional Questions" form. In Table VIII, results from a Pearson correlation analysis comparing the number of cigarettes smoked with 3-EP, benzene, styrene, and TVOC concentrations are reported for the smoking homes. In addition to the "cigarettes observed" variable, two normalized variables, "cigarettes observed/room" and "cigarettes observed/room excluding bathrooms" are included. The normalized variables are included to account for differences in home size. Ideally, the normalization should be performed with the actual house volume and/or measured ventilation rates; however, these variables were neither measured nor estimated.

Of the four analytes, only 3-ethenylpyridine, the vapor phase ETS marker, exhibits a

statistically significant correlation with any of the smoking activity variables. The cigarettes observed variable ($r=0.29$), is not significantly correlated with 3-EP; however, the two normalized variables, cigarettes observed/room ($r=0.40$) and cigarettes observed/room excluding bathrooms ($r=0.40$), are significantly correlated at $p \leq 0.05$. Even though two of the three correlations are significant, the correlation coefficient, r, is small; however, several factors may affect this relationship: (1) room area variability; (2) house volume variability; (3) air exchange rate variability; (4) sink source variability; (5) counting or reporting errors; and (6) ETS cigarette delivery variability.

The remaining selected analytes, benzene ($r=0.018$), styrene ($r=-0.03$), and TVOC ($r=-0.04$), are not significantly correlated with the number of cigarettes observed at $p \leq 0.05$ or $0.05 < p \leq 0.10$. Benzene ($r=0.16$), styrene ($r=0.01$), and TVOC ($r=0.02$) are not significantly correlated with the number of cigarettes observed/room. In addition, benzene ($r=0.18$), styrene ($r=0.01$), and TVOC ($r=0.01$) are not significantly correlated with the number of cigarettes observed/room excluding bathrooms. Although benzene and styrene are elevated in smoking homes versus nonsmoking homes as determined by the Wilcoxon Rank Sum Test (Table I), apparently several other sources contribute significantly to this elevation. In fact, benzene, styrene, and TVOC concentrations exhibit poorer correlation coefficients and p values with the normalized smoking activity variables than with the raw smoking activity variable, whereas the normalized smoking activity variables improve the correlation coefficients and p values with respect to 3-EP.

Benzene Source Identification. In order to evaluate benzene sources, a number of potential indoor and outdoor sources of benzene were identified and selected from questionnaire responses: smoking husband, gas or oil heat, fireplace, gas jets (logs), wood stove, gas range, cooking dinner, kerosene heater, type of home (apartment or single-family dwelling), attached garage, storing chemicals in the home, and distance from a highway. Benzene contributions from each of these potential sources were evaluated by conducting a Wilcoxon Rank Sum Test on the levels of benzene observed in homes where a given source was present, compared to homes in which it was not present. A Kruskal-Wallis Test was conducted for six levels of the distance from highway variable. The p values for these tests are given in Table IX. Only smoking husband, gas heat, gas or oil heat, and electric heat sources are significant at $p \leq 0.05$. Of the 49 homes, 38 used gas heat, 9 used electric heat, one used oil heat, and one reported geothermal heat. Thus, the set of homes heated with gas or oil is nearly identical to those heated with gas, and both are approximately the complement of those that used electricity. It should be noted that only one home reported the use of gas jets (logs), a woodstove, or a kerosene heater, and only two reported the use of a fireplace during the pump period. Therefore, tests of these potential sources have very little statistical power. Of the 17 potential sources, only smoking husband and gas vs. electric heat are sources that result in significantly different levels of benzene.

Benzene Regression Model. Using the dichotomous variables addressed in the previous section (Table IX) and the continuous variables related to the number of cigarettes

smoked during the three-hour period, a regression model was developed in an attempt to account for the variables resulting in elevated benzene concentrations. In Table X, results of a Pearson correlation analysis for the continuous variables and a benzene regression analysis are presented. For purposes of discussion, the variables are classified as either "ETS" or "non-ETS" variables. For the non-ETS variables, the regression model explains 23% of the total variance encountered; however, the overall model is insignificant (p=0.73). Adding the dichotomous ETS variable, smoking husband, to the non-ETS variables improves the model slightly by explaining 28% of the total variance; however, the overall model remains insignificant (p=0.61). The incremental addition of the smoking husband variable to the model is also insignificant at p=0.15. Removing this variable and adding the continuous ETS variable, cigarettes observed/room excluding bathrooms, to the non-ETS variables improves the model slightly by explaining 27% of the total variance; however, the overall model remains insignificant (p=0.66). The incremental addition of cigarettes observed/room excluding bathrooms is also insignificant at p=0.21. Removing this variable and adding the continuous ETS variable, cigarettes observed/room, to the non-ETS variables improves the model slightly by explaining 26% of the total variance; however, the overall model remains insignificant (p=0.68). The incremental addition of cigarettes observed/room is also insignificant at p=0.22. Removing this variable and adding the continuous ETS variable, cigarettes observed, to the non-ETS variables improves the model slightly by explaining 27% of the total variance; however, the overall model remains insignificant (p=0.68). The incremental addition of cigarettes observed is also insignificant at p=0.24. For the dichotomous and

continuous ETS variables alone, the regression model explains 8% of the total variance encountered; however, the overall model is insignificant ($p=0.42$). Finally, adding all of the ETS and non-ETS variables to the model explains 30% of the total variance encountered; again, the overall model is insignificant ($p=0.78$). In conclusion, neither the ETS nor the non-ETS variables separately or combined yield significant model regression coefficients with respect to the benzene contributions. The non-ETS variables account for more of the variance ($r^2=0.23$) than the ETS variables ($r^2=0.08$). Obviously, the model's ability to identify and account for benzene sources other than gas heat and smoking husbands is weak. Apparently, the sum total of all other unidentified benzene sources exerts an overall greater effect on benzene concentrations than the variables applied in the model.

ETS Apportionment. The statistical techniques described thus far such as ranking, correlation analysis, factor analysis, and regression modeling are limited in their ability to determine true apportionment of VOC sources. For example, absolute benzene and styrene concentrations are higher in the 25 smoking homes than in the 24 nonsmoking homes, but correlation analysis and factor analysis methods indicate that the benzene and styrene concentrations are not correlated with the ETS marker concentrations (Tables II and III). In addition, benzene and styrene concentrations are not correlated with the other smoking-related indicators such as the number of cigarettes observed during the sample period (Table VIII). Finally, the benzene regression model is unable to predict benzene level with reasonable confidence given the input source variables. In a situation

[illegible] particular VOC, [illegible] as benzene, [illegible] generated from multiple, [illegible]
[illegible] technique [illegible] needed to identify [illegible] contribution to the total analyte concentration. Daisey et al. (1991) presented a technique using model estimates of the contribution of ETS to VOCs and suggested the possibility of an apportionment technique using "gas-phase nicotine or some other unique tracer of ETS and the ratio of nicotine (or tracer) to VOC in ETS." In an earlier study (Heavner et al. 1992), an apportionment technique was described in which the ratio of a vapor phase ETS marker, 3-ethenylpyridine, to selected analytes was determined in an environmental chamber across a range of air exchange rates (0 to 2 per hour) with ETS generated from a 1R4F test cigarette (University of Kentucky, Lexington, KY) as the sole source of VOCs. Then, the percentage of the VOC attributable to ETS (%Analyte$_{ETS}$) was calculated based on the 3-EP/Analyte ratio from field determinations. The utility of this technique was tested in four smoking and four nonsmoking homes. (Heavner et al. 1992)

In Table XI, apportionment results from each of the 25 smoking homes with summary statistics are presented for eight selected analytes: TVOC, benzene, styrene, pyridine, 2-picoline, 3-picoline, 4-picoline, and 3-ethylpyridine. Because the data are not normally distributed, the medians are more meaningful than the means in comparing results. For TVOC, the median percentage attributable to ETS is 5.5%, ranging from 0.0% to 25.8%. For benzene, the median percentage attributable to ETS is 13.2%, ranging from 0.0% to 63.2%. For styrene, the median percentage attributable to ETS is 12.6%, ranging from 0.0% to 58.1%. For the nitrogenous bases, pyridine, 2-picoline, 3-picoline, 4-picoline, and

[illegible]ethylpyridine, the median percentages attributable to ETS are 40.7%, 67.1%, 90.1%, [illegible].2%, and 62.0%, respectively. For all of the nitrogenous bases, percentages attributable to ETS are greater than 100% in some of the homes. In these homes, the absolute concentrations (in $\mu g/m^3$) are extremely low compared to the concentrations used to determine the 3-EP/Analyte ratios in the environmental chamber. Consequently, small differences in the field determinations may result in apportionment percentages greater than 100%. Rather than arbitrarily limiting these values at 100%, the uncorrected values are presented to illustrate the difficulty in determining apportionment percentages when the absolute concentrations approach the limit of quantitation for a specific analyte. Overall, the ETS apportionment technique demonstrates the multisource nature of VOCs in indoor air environments and provides one quantitative estimate of the percentage of selected VOCs that originate from smoking activity.

Furthermore, the $\%Analyte_{ETS}$ determinations may be used to calculate the absolute concentration of analytes originating from ETS and from all other sources combined. In Figure 1, the "Total" concentrations and the "ETS" concentrations are presented in a histogram format for four of the analytes (TVOC, benzene, styrene, and 3-picoline) from the 24 nonsmoking and the 25 smoking homes. The results are paired and ranked in increasing order of "Total" concentration. TVOCs are not calculated for three of the 49 homes due to GC/MSD instrument problems resulting in the loss of data from the back cartridges. The histograms graphically illustrate the ETS contribution to the total concentration and place into perspective the relationship between the portion originating

from ETS and the portion originating from other, non-ETS sources. In general, these results demonstrate that even in the absence of smoking activity, substantial levels of TVOCs and VOCs are generated. ETS does contribute to the total concentration of specific VOCs; however, the elimination of ETS as a source has minimal impact on the total concentration with the exception of the nitrogenous bases. Most importantly, ETS contributes relatively little to the aggregate VOC indicator, the TVOC concentration.

Conclusions

In this study, the levels of VOCs measured during a three-hour period in 25 smoking homes and 24 nonsmoking homes have been discussed and related to a number of multiple indoor air sources. Of the 29 analytes, one VOC is elevated in the nonsmoking homes, seven VOCs are elevated in the smoking homes, and 21 VOCs are not statistically different. Storing gasoline in a garage or basement contributes to elevated levels of eight aromatic hydrocarbons. Using mothballs in the home contributes to elevated levels of 1,4-dichlorobenzene. Wearing dry-cleaned clothes contributes to elevated levels of tetrachloroethylene. Using stain remover or furniture polish contributes to elevated levels of limonene. Although benzene and styrene are elevated in smoking homes, these VOCs are not significantly correlated with the number of cigarettes smoked or the proposed vapor phase ETS marker, 3-ethenylpyridine, indicating that a number of other unidentified sources contribute to this elevation. Based on nonparametric tests of 17 source input variables, gas heat and smoking husband are

identified as source variables contributing to indoor air benzene levels; however, a regression analysis model indicates that the ETS variables account for only 8% of the total variance encountered. The non-ETS variables account for 23% of the total variance encountered. The technique of ETS apportionment based on 3-ethenylpyridine/VOC ratios appears promising, but further investigation is required in order to determine 3-EP ratios for existing market cigarette brands. Nonetheless, this technique is potentially useful in assessing the portion of indoor air VOCs attributable to ETS in environments where multiple sources are responsible for contributing to the total concentration of a specific analyte. The median percentages of TVOC, benzene, and styrene attributable to ETS in smoking households are 5.5%, 13.2%, and 12.6%, respectively.

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Table I. VOC summary statistics for smoking and nonsmoking homes.

Compound	Nonsmoking Homes ($\mu g/m^3$)						Smoking Homes ($\mu g/m^3$)						Wilcoxon
	n	Mean	S.D.	Median	Minimum	Maximum	n	Mean	S.D.	Median	Minimum	Maximum	
n-Nonane	22	3.00	2.60	2.01	0.51	8.95	25	3.84	4.18	2.41	0.54	16.67	[illegible]
Benzene	24	3.86	4.05	2.42	1.29	18.96	25	5.54	5.13	4.03	0.98	26.96	[illegible]
n-Decane	24	5.07	4.95	2.36	1.46	16.86	25	4.99	9.00	2.75	0.00	46.02	[illegible]
Trichloroethylene	24	1.84	2.39	1.05	0.00	9.08	25	0.66	1.04	0.00	0.00	3.41	[illegible]
Tetrachloroethylene	24	1.24	1.46	0.70	0.00	5.13	25	0.39	0.96	0.68	0.00	3.78	[illegible]
Toluene	24	19.25	13.11	13.65	3.01	47.43	25	27.78	22.17	23.79	4.42	118.20	[illegible]
1,2-Dichloroethane	24	0.00	0.00	0.00	0.00	0.00	25	0.02	0.08	0.00	0.00	0.40	[illegible]
n-Undecane	24	3.86	4.64	1.99	0.14	17.19	25	14.50	49.93	1.81	0.56	249.68	[illegible]
Ethylbenzene	24	3.35	4.87	2.12	0.36	25.39	25	3.07	3.57	2.21	0.82	19.45	[illegible]
p-Xylene	24	3.63	6.14	2.08	1.01	31.78	25	3.64	4.37	2.34	0.80	22.42	[illegible]
m-Xylene	24	7.24	9.30	4.45	1.02	47.21	25	7.57	7.58	5.71	0.16	35.96	[illegible]
Isopropylbenzene	24	0.46	3.05	0.26	0.00	3.11	25	0.45	0.66	0.27	0.00	3.23	[illegible]
o-Xylene	24	4.21	6.61	2.43	1.13	34.24	25	4.21	5.13	2.57	1.16	25.41	[illegible]
Pyridine	24	0.67	0.53	0.66	0.01	1.86	25	2.34	2.03	1.97	0.00	8.59	[illegible]
n-Dodecane	24	1.63	1.39	1.34	0.32	4.50	25	7.34	25.21	1.43	0.19	127.90	[illegible]
Limonene	24	17.81	11.27	13.82	3.45	41.98	25	23.15	15.94	20.77	5.68	57.39	[illegible]
n-Propylbenzene	24	1.06	1.91	0.60	0.23	9.88	25	0.93	1.26	0.52	0.00	5.20	[illegible]
2-Picoline	24	0.07	0.18	0.00	0.00	0.67	25	0.45	0.35	0.38	0.00	1.55	[illegible]
1,3,5-Trimethylbenzene	24	1.85	1.85	1.12	0.41	15.29	25	1.92	2.33	1.02	0.23	8.81	[illegible]
Styrene	24	1.47	1.02	1.30	0.43	4.96	25	2.11	1.20	1.92	0.49	7.02	[illegible]
n-Tridecane	24	1.41	0.55	1.38	0.38	2.73	25	2.67	3.95	1.26	0.28	15.59	[illegible]
3-Picoline	24	0.14	0.16	0.09	0.00	0.51	25	0.68	0.56	0.58	0.00	2.40	[illegible]
4-Picoline	24	0.09	0.19	0.00	0.00	0.69	25	0.16	0.26	0.00	0.00	0.94	[illegible]
n-Butylbenzene	24	0.27	0.36	0.16	0.00	1.39	25	0.34	0.41	0.26	0.00	1.54	[illegible]
1,2,3-Trimethylbenzene	24	1.76	2.10	1.29	0.37	10.91	25	2.19	2.69	1.18	0.42	10.11	[illegible]
3-Ethylpyridine	24	0.03	0.10	0.00	0.00	0.46	25	0.18	0.24	0.00	0.00	0.68	[illegible]
1,4-Dichlorobenzene	24	3.45	6.50	0.30	0.00	25.39	25	10.22	30.24	0.16	0.00	116.23	[illegible]
3-Ethenylpyridine	24	0.08	0.19	0.00	0.00	0.57	25	1.28	1.31	0.95	0.00	5.58	[illegible]
TVOC	22	289	251	[illegible]	56	921	24	858	2825	252	79	14097	[illegible]

* Significant at p ≤ 0.05

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Table II. Pearson correlation matrix for VOC analytes in all homes (smoking and nonsmoking).

Compound	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)
(1) n-Nonane			●				●	●				●		□	●				●					●	●	
(2) Benzene						●			●	●	●		●				●	●	●	□				●	●	
(3) n-Decane	●																							●		
(4) Trichloroethylene																									□	
(5) Tetrachloroethylene						●																				
(6) Toluene		●			●				□	□			□					●			●					●
(7) 1,2-Dichloroethane	●							●				●		●	●	●		●			●	●		●	●	●
(8) n-Undecane	●						●					●		●	●	●		●			●	□		●	●	●
(9) Ethylbenzene		●				□				●	●	●	●				●		●	●				●	●	
(10) p-Xylene		●				□			●		●	●	●				●		●	●				●	●	
(11) m-Xylene		●							●	●		●	●				●		●	●				●	●	
(12) Isopropylbenzene	●						●	●	●	●	●		●	●	●		●		●		□			●	●	
(13) o-Xylene		●				□			●	●	●	●					●		●	●				●	●	
(14) Pyridine	□						●	●				●			●	●		●		●		●	●			●
(15) n-Dodecane	●						●	●				●		●		□		●			●	●		●	●	●
(16) Limonene							●	●						●	□			●								●
(17) n-Propylbenzene		●							●	●	●	●	●						●	●				●	●	
(18) 2-Picoline		●				●	●	●						●	●	●				●		●	●			●
(19) 1,3,5 Trimethylbenzene	●	●							●	●	●	●	●				●			●				●	●	
(20) Styrene		□							●	●	●		●	●			●	●	●			●			●	
(21) n-Tridecane						●	●	●				□			●									□		●
(22) 3-Picoline							●	□						●	●			●		●			●			●
(23) 4-Picoline														●				●				●				●
(24) n-Butylbenzene	●	●	●				●	●	●	●	●	●	●		●		●		●		□				●	
(25) 1,2,3-Trimethylbenzene	●	●	□				●	●	●	●	●	●	●		●		●		●	●				●		
(26) 3-Ethylpyridine	●	□				●	●	●						●	●	●		●			●	●	●			
(27) 1,4-Dichlorobenzene	●		●																							
(28) 3-Ethenylpyridine														●		□		●				●	●			●
(29) TVOC	●						●	□				●		●	●	●		●	□		●	●	□	●	●	●

● Significant at p ≤ 0.05 □ Significant at 0.05 < p ≤ 0.10

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Table III. Factor analysis results for VOC analytes in all homes (smoking and nonsmoking).

	Correlation Between Variables and Factors ($r \geq 0.50$)						
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Variance Explained by Each Factor	7.912	6.482	4.936	2.063	1.640	1.279	1.195
Compound & Correlation Coefficient	Benzene 0.61	*n*-Nonane 0.51	Pyridine 0.71	*n*-Nonane 0.68	Tetrachloroethylene 0.74	Trichloroethylene -0.53	Styrene 0.65
	Ethylbenzene 0.91	1,2-Dichloroethane 0.98	2-Picoline 0.92	*n*-Decane 0.91	Toluene 0.82	Limonene 0.73	
	p-Xylene 0.97	*n*-Undecane 0.97	3-Picoline 0.92	1,4-Dichlorobenzene 0.80			
	m-Xylene 0.98	Isopropylbenzene 0.59	4-Picoline 0.81				
	Isopropylbenzene 0.59	*n*-Dodecane 0.98	3-Ethylpyridine 0.84				
	o-Xylene 0.99	*n*-Tridecane 0.51	3-Ethenylpyridine 0.89				
	n-Propylbenzene 0.98	TVOC 0.97					
	1,3,5-Trimethylbenzene 0.96						
	n-Butylbenzene 0.77						
	1,2,3-Trimethylbenzene 0.82						

SENT BY: ; 8- 1-94 ; 17:13 ; ETS/BGTC→ +49 221 1646 858;#43

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SENT BY: ; 8- 1-94 ; 17:15 ; ETS/BGTC→ +49 221 1646 658;#45

	Wilcoxon Rank Sum p-Value		
	Nonsmoking Homes	Smoking Homes	All Homes
Air Freshener	0.08	0.62	0.58
Liquid Bleach	0.41	0.93	0.53
Furniture Polish	0.08	0.37	0.05*
Hair Spray	0.009*	0.92	0.06
Kitchen Cleaner	0.75	0.40	0.30
Window Cleaner	0.73	0.77	0.46
Fingernail Polish	0.41	0.68	0.75
Carpet Freshener	0.50	0.73	0.49
Carpet Cleaner	0.66	0.30	0.25
Stain Remover	0.10	0.05*	0.01*
Perfume	0.02*	0.39	0.21
Bug Killer	Not Used	0.15	0.19
Exposed to ETS During the "Pump Period"	---	0.83	0.32

* Significant at $p \leq 0.05$

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Table V. Wilcoxon Rank Sum Test results in all homes (smoking and nonsmoking): (a) trichloroethylene/tetrachlorethylene and wearing clothes that were dry-cleaned in the week prior to wearing the sampler; and (b) 1,4-dichlorobenzene and using mothballs in the home.

Compound	Wilcoxon Rank Sum p Value		
	Nonsmoking Homes	Smoking Homes	All Homes
(a) Trichloroethylene	0.66	0.14	0.18
Tetrachloroethylene	0.11	0.18	0.03*
(b) 1,4-Dichlorobenzene	0.20	0.12	0.03*

* Significant at $p \leq 0.05$

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SENT BY: ; 8- 1-94 ; 17:17 ; ETS/BGTC→ +49 221 1648 658;#47

	Nonsmoking Homes	Smoking Homes	All Homes
Benzene	0.88	0.48	0.70
Toluene	0.09	0.98	0.25
Ethylbenzene	0.12	0.18	0.05*
p-Xylene	0.17	0.04*	0.03*
m-Xylene	0.17	0.08	0.06
Isopropylbenzene	0.10	0.17	0.02*
o-Xylene	0.03*	0.03*	0.004*
n-Propylbenzene	0.08	0.05*	0.003*
1,3,5-Trimethylbenzene	0.12	0.10	0.01*
Styrene	0.90	0.89	0.40
n-Butylbenzene	0.21	0.23	0.12
1,2,3-Trimethylbenzene	0.13	0.20	0.04*

* Significant at $p \leq 0.05$

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SENT BY: ; 8- 1-94 ; 17:18 ; ETS/BGTC→ +49 221 1646 858;#48

[illegible]	Correlation Coefficient (r)	p Value
5-Day Nicotine "Light" Use Area Fixed Monitor	0.46	0.0203*
5-Day Nicotine "Heavy" Use Area Fixed Monitor	0.84	0.0001*
5-Day Nicotine Average Fixed Monitors	0.78	0.0001*
5-Day Nicotine Personal Monitor	0.84	0.0001*
5-Day 3-Ethenylpyridine "Light" Use Area Fixed Monitor	0.88	0.0001*
5-Day 3-Ethenylpyridine "Heavy" Use Area Fixed Monitor	0.88	0.0001*
5-Day 3-Ethenylpyridine Average Fixed Monitors	0.89	0.0001*
5-Day 3-Ethenylpyridine Personal Monitor	0.72	0.0001*
"Demo" Salivary Cotinine	0.61	0.0012*
Monday Salivary Cotinine	0.36	0.0772
Wednesday Salivary Cotinine	0.71	0.0001*
Friday Salivary Cotinine	0.64	0.0006*
Average Salivary Cotinine	0.62	0.0009*

* Significant at $p \le 0.05$

2026224078

Table VIII. Pearson correlation analysis results comparing the number of cigarettes smoked with 3-ethenylpyridine, benzene, styrene, and [illegible] concentrations in smoking homes.

Cigarettes	3-Ethenylpyridine		Benzene		Styrene		TVOC	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
Observed	0.29	0.15	0.18	0.39	-0.03	0.87	-0.04	0.85
Observed/Room	0.40	0.049*	0.16	0.46	0.01	0.96	0.02	0.92
Observed/Room Excluding Bathrooms	0.40	0.049*	0.18	0.40	0.01	0.97	0.01	[illegible]

* Significant at $p \leq 0.05$

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SENT BY: ; 8- 1-94 ; 17:19 ; ETS/BGIC→ +49 221 1648 658;#49

	n	[illegible] ($\mu g/m^3$)	[illegible] ($\mu g/m^3$)	n	Mean ($\mu g/m^3$)	Median ($\mu g/m^3$)	
Smoking Husband	24	5.54	4.03	25	3.86	2.42	0.006*
Gas Heat	38	5.33	3.54	11	2.59	2.43	0.01*
Electric Heat	9	2.55	2.43	40	5.20	3.50	0.02*
Gas or Oil Heat	39	5.25	3.51	10	2.62	2.46	0.02*
Used Fireplace	2	5.91	5.91	47	4.66	3.22	0.18
Used Gas Jets	1	7.79	7.79	48	4.65	3.24	0.19
Used Wood Stove	1	1.81	1.81	48	4.78	3.36	0.19
Oil Heat	1	2.28	2.28	48	4.77	3.36	0.42
Distance from Highway†	—	---	---	--	—	—	0.45
Cooked Dinner	36	4.54	3.36	13	5.19	3.22	0.46
Gas Range	12	5.70	3.15	37	4.40	3.47	0.65
Used Kerosene Heater	1	2.72	2.72	48	4.76	3.36	0.70
Single Family Home	44	4.82	3.24	5	3.83	3.47	0.77
Store Chemicals in the Home	38	5.02	3.35	11	3.65	3.25	0.79
Attached Garage	35	5.11	3.25	14	3.72	3.26	0.79
Used Gas Range	8	6.25	3.15	41	4.42	3.47	0.86
Geothermal Heat	1	3.22	3.22	48	4.75	3.36	0.97

†Kruskal-Wallis Test (Six levels) * Significant at $p \leq 0.05$

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Table X. Pearson correlation analysis and regression model results comparing ETS and non-ETS sources with benzene concentrations all homes.

Source Variables Included in Regression Model	Pearson Correlation for "ETS" Variables (n=49)		Regression Analysis		
	Correlation Coefficient (r)	p Value	Model r^2	p Value Overall	p Value Incremental
All "Non-ETS" Variables	---	---	0.23	0.73	---
All "Non-ETS" Variables + Smoking Husband	0.18	0.21	0.28	0.61	0.15
All "Non-ETS" Variables + Cigarettes Observed/Room Excluding Bathrooms	0.22	0.12	0.27	0.66	0.21
All "Non-ETS" Variables + Cigarettes Observed/Room	0.21	0.15	0.26	0.68	0.22
All "Non-ETS" Variables + Cigarettes Observed	0.22	0.12	0.27	0.67	0.24
All "Non-ETS" + All "ETS" Variables	---	---	0.30	0.78	---
All "ETS" Variables	---	---	0.08	0.42	0.57

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Table XI. ETS apportionment results for selected analytes in smoking homes.

Home #	Apportionment %							
	$TVOC_{ETS}$	$Benzene_{ETS}$	$Styrene_{ETS}$	$Pyridine_{ETS}$	2-$Picoline_{ETS}$	3-$Picoline_{ETS}$	4-$Picoline_{ETS}$	3-$Ethylpyridine_{ETS}$
1	0.59	3.35	2.52	5.84	15.65	60.23	12.76	ND
2	2.97	5.47	10.04	18.02	28.40	144.43	31.15	ND
3	5.48	25.73	23.92	39.65	78.60	134.88	105.72	80.9[illegible]
7	1.07	2.04	1.58	12.65	21.74	150.58	ND	[illegible]
8	20.87	39.03	32.96	41.66	88.52	123.10	ND	94[illegible]
9	8.29	26.24	24.74	39.18	73.88	94.77	85.25	65[illegible]
10	0.96	4.17	4.58	5.62	18.78	34.75	6.96	[illegible]
12	0.00	0.00	0.00	0.00	ND	ND	ND	[illegible]
13	2.54	27.63	30.60	45.98	74.14	102.28	183.74	[illegible]
15	14.66	41.26	39.36	80.01	84.40	90.08	24.43	[illegible]
18	0.83	4.67	6.94	19.94	15.01	18.72	8.36	[illegible]
20	0.06	10.23	5.58	6.99	15.31	19.78	10.21	[illegible]
27	8.95	33.28	19.30	78.68	67.08	88.40	ND	[illegible]
31	19.74	29.38	18.19	58.41	68.26	106.18	ND	[illegible]
33	18.54	40.21	43.51	28.84	82.41	91.63	58.33	62[illegible]
36	6.98	17.63	12.63	70.81	41.92	27.76	ND	N[illegible]
38	7.49	13.23	9.36	22.66	47.91	57.36	ND	N[illegible]
40	10.86	9.39	11.00	99.66	66.38	70.47	ND	N[illegible]
41	ND	5.11	43.82	120.72	65.61	113.25	ND	46[illegible]
42	1.58	9.17	6.02	302.13	ND	79.43	ND	N[illegible]
43	5.32	33.64	26.56	108.34	70.56	88.54	45.02	N[illegible]
48	25.78	45.33	44.04	53.54	102.48	113.76	ND	N[illegible]
49	0.00	0.00	0.00	0.00	ND	0.00	ND	N[illegible]
50	16.75	63.17	58.14	115.79	80.50	100.03	43.28	95[illegible]
54	0.00	0.00	0.00	ND	ND	ND	ND	N[illegible]
Mean	7.51	19.57	19.01	57.30	57.50	83.06	51.27	58[illegible]
S.D.	7.87	17.56	16.92	64.38	27.99	41.43	52.15	36[illegible]
Median	5.48	13.23	12.63	40.66	67.08	90.08	37.22	62[illegible]
Minimum	0.00	0.00	0.00	0.00	15.01	0.00	6.96	9.0[illegible]
Maximum	25.78	63.17	58.14	302.13	102.48	150.58	183.74	103[illegible]

ND = None Detected

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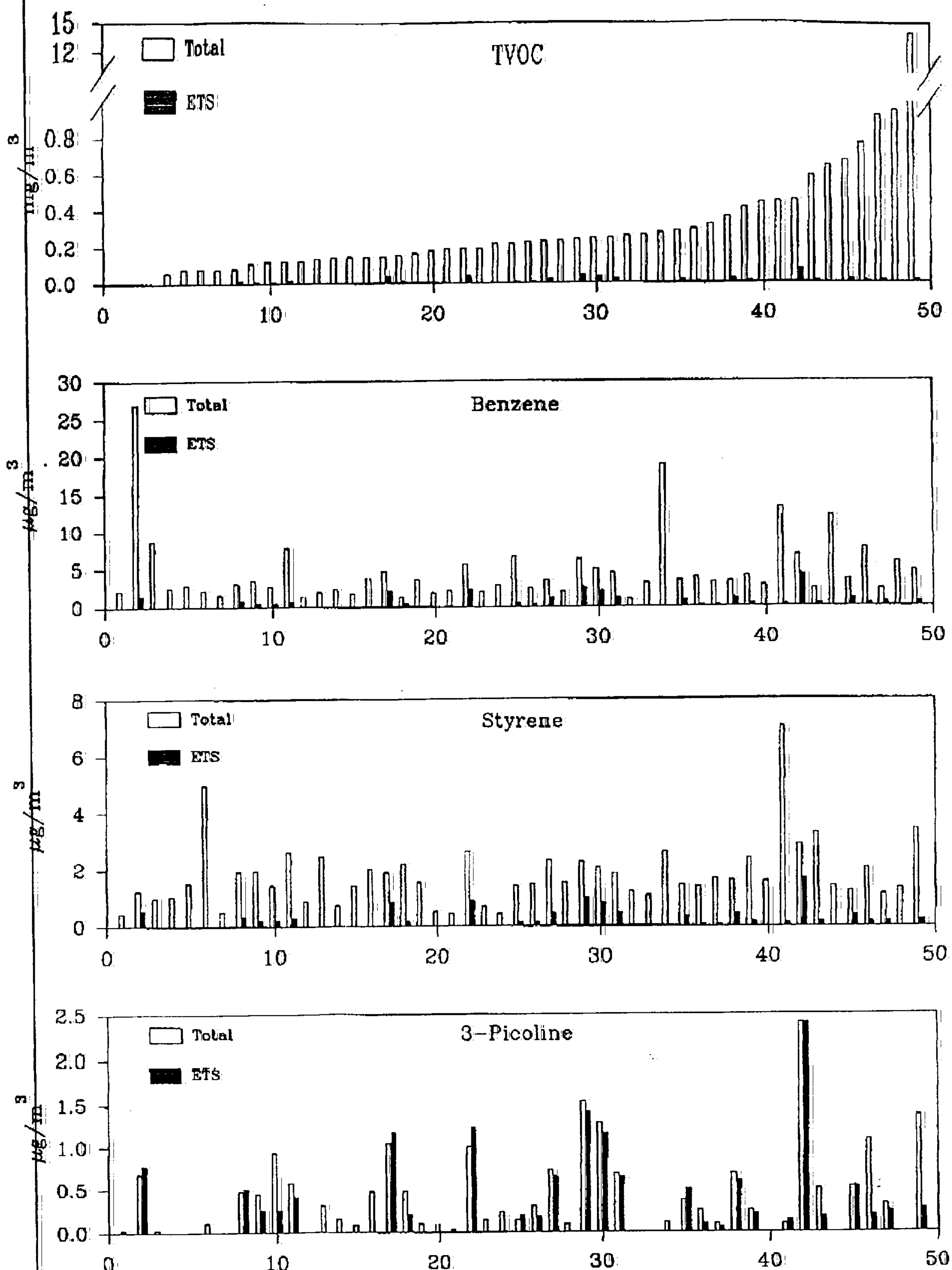
List of Figures


TVOC
Total
ETS
mg/m3
15
12
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Benzene
Total
ETS
µg/m3
30
25
20
15
10
5
0
Styrene
Total
ETS
µg/m3
8
6
4
2
0
3-Picoline
Total
ETS
µg/m3
2.5
2.0
1.5
1.0
0.5
0.0

2026224084

ASSESSMENT OF PERSONAL EXPOSURES TO ENVIRONMENTAL TOBACCO SMOKE IN BRITISH NON SMOKERS

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ABSTRACT

Environmental Tobacco Smoke (ETS) exposure of 255 non-smoking subjects was assessed by several methods. Each subject wore a personal air sampler for 24 hours, answered a questionnaire about air quality and ETS exposure, and provided saliva samples for cotinine analysis before and after the monitoring period. The study was conducted in the Leeds and Harrogate areas of northern England.

Median exposure to ETS particles was below the detection limit of 4 $\mu g\ m^{-3}$. Median exposure to nicotine was 0.5 $\mu g\ m^{-3}$ and median saliva cotinine levels were 0.7 $ng\ mL^{-1}$ and 0.6 $ng\ mL^{-1}$ for the pre and post-cotinine samples. Median exposure to particles from all sources was 142 $\mu g\ m^{-3}$. Approximately 80% of subjects assessed their ETS exposure as 'none' or 'low'.

On average, the home made the greatest contribution to ETS exposure, followed by leisure, and then work. Travel was a minor contributor to exposure.

Overall, subjects with a partner who smokes were exposed to more ETS than subjects with no partner or a non-smoking partner. However there was considerable overlap in the exposures of individuals within these groups.

Where subjects assessed their ETS exposure as 'none' or 'low', this was in general supported by the direct measurements of exposure. However, for exposure assessed as 'moderate' or 'high' there was a wide range in the corresponding direct measurements.

There was a moderate correlation between exposure to nicotine and exposure to ETS particles (R^2=0.66), but poor correlation between nicotine exposure and saliva cotinine levels (R^2=0.07 for pre and R^2=0.13 for post-cotinine samples).

Overall, ETS made only a small (median 2.5%) contribution to particles from all sources as collected by the personal monitor. Exposure to ETS particles did not correlate (R^2=0.04) with exposure to particles from all sources.

Out of 327 volunteers recruited as non-smokers for this study, 53 (16%) were identified as likely smokers by saliva cotinine levels or detailed questioning.

INTRODUCTION

Two main approaches have been used in the past to assess whether there is any risk associated with exposure to ETS. One is based on epidemiology and the other based on the quantities of smoke constituents to which non-smokers are exposed.

A criticism of published epidemiological studies of ETS is that almost all failed to include a direct measurement of exposure level (Coultas et al. 1989). Although spousal smoking has frequently been used as an index of exposure in these studies, the accuracy of this approach has been questioned (Koo et al. 1987). Therefore, it is important to determine how well reported spousal smoking correlates with directly measured exposure. It is also important to determine how well ETS exposure can be predicted by questionnaire or by measurements of saliva cotinine since these approaches are also used as an alternative to direct measurements of exposure. In the case of cotinine measurements consideration must be given to threshold levels (Etzel 1990) in order to exclude smokers from ETS exposure evaluation. There have been various ideas on what defines a regular smoker. The EPA for example defined regular smokers as those having more than 30% of the average cotinine level found for smokers.

Most of the information about the quantities of smoke constituents to which non-smokers may be exposed is based on fixed-site measurements of ETS levels in locations such as homes, offices and restaurants together with assumptions about the time people are thought to spend in these locations. There have been numerous such studies (reviewed by

Guerin et al. 1992) particularly in the USA, but these do not characterise properly the range of ETS exposure experienced by non-smokers over a period of time.

There have, until recently, been few attempts to measure directly the exposure of people as they go about their normal lives, even though this approach should provide more realistic results than those calculated from ETS levels in locations. The use of personal monitoring has been common practice in the industrial hygiene field for many years, but it is only recently that the analytical methodology has been refined sufficiently to allow ETS measurements to be carried out by this approach. When personal monitoring is used subjects can be monitored over set periods, such as 24 hours or longer. It could be assumed that the monitoring period is typical of a subject's normal exposure but it is preferable to establish that this is the case by questionnaire.

The levels of both nicotine and ETS particles have been determined in several studies of locations, but personal monitoring studies have tended to measure nicotine and not particles. In view of the limitations of nicotine as a marker for ETS (Guerin et al. 1992), there clearly is a need for personal monitoring studies in which nicotine and ETS particles are measured simultaneously. It is now possible to estimate the ETS contribution to particles from all sources (PAS) by a number of methods. Measurements using ultra violet light (UVPM) and fluorescence (FPM) and the determination of solanesol (SolPM) are all used in this study.

UVPM measurements and, to a lesser extent FPM measurements, are believed to overestimate ETS contributions to total particles because of the presence of interfering

compounds. Solanesol is a constituent of Tobacco and other members of the Solanacae family, e.g potatoes. It is found in the particulate phase of ETS and its presence in air is likely to be derived only from tobacco smoke and would not be expected to overestimate ETS particles.

In this study, the 24-hour exposure to ETS of a group of UK non-smokers was assessed by personal monitoring. Subjects were asked detailed questions about their smoking history and exposure to ETS, and saliva samples were taken for cotinine measurements. Subjects were asked to assess their overall exposure to ETS. These assessments could then be compared to measurements made directly using the monitor. Relative contributions from the home, work, leisure and travel to overall ETS exposure were assessed by direct measurements in conjunction with questionnaire information. In addition, spousal smoking, questionnaires and saliva cotinine levels were compared with personal monitoring as methods of assessing ETS exposure. Comparison of the methods of assessing ETS exposure was also made in order to determine the extent of any correlation. The weather conditions were also monitored and recorded throughout the study period.

Some researchers refer to 'cigarette equivalents' when assessing the risk of ETS exposure to non smokers. However, mainstream smoke (that inhaled) is not chemically identical to ETS smoke or exhaled smoke. Comparison of the quantities of ETS exposure with cigarette yields has been made in this study, but only to allow the magnitude of the exposure to be put into perspective.

Misclassification occurs in ETS studies when smokers report themselves to be non-smokers or vice versa. In this study subjects were regarded as misclassified and they were excluded from ETS exposure evaluation on the basis of their saliva cotinine levels. The rejection criteria used were based on published saliva cotinine levels for smokers and non-smokers (Etzel 1990).

METHODS

Recruitment of subjects

Potential subjects for use in this study were randomly selected from an existing data base of 15,000 volunteers held at Besselaar Clinical Research Unit, Leeds, England. They were contacted by letter and asked to take part in a general air quality study. Those who volunteered had to complete a recruitment questionnaire which included a question about their smoking status. Only those volunteers who confirmed that they were non-smokers were considered, from which 327 were used on this study.

To avoid the possibility of influencing their behaviour, subjects were told that the purpose of the study was to assess Air Quality and were not informed that it was related to ETS exposure.

The Monitoring Session

The personal monitors were delivered by an investigator (a member of Hazleton Europe's scientific staff) to each subject on the appointment date, and the use of the pump and the wearing of the monitor was explained. The subjects were asked to wear the monitor at all times throughout the 24 hour test period except when in bed or bathing/showering, when they were asked to place the monitor nearby in a vertical position. Each subject was asked to complete an activity diary over the 24 hour period and to record observations of general air quality including the presence of tobacco smoke.

At the start of the session the caps were removed from the personal monitor and the pump stroke counts recorded. The pump was switched on by the investigator, and a saliva sample (pre-sample) taken.

After 24 hours the pump was switched off and the pump stroke counts recorded. A comprehensive questionnaire was completed by the investigator, with the subject referring to the diary as necessary. Finally, a saliva sample (post-sample) was taken.

The pump stroke counts were used to confirm that the pump had operated continuously. The pump flow rate was measured before and after each monitoring session.

Collection of Saliva Samples

Dental swabs sealed in hygienic vials (salivettes, Sarstedt, Leicester, England), were used to collect saliva samples. By removing the lid and tilting the vial to the lips, the subject could transfer the swab to the mouth without touching it. After chewing for a timed 1.5 minutes, the swab was returned to the vial using the tongue. The cap was then replaced and the vial stored in a freezer (-20°C) until required for analysis.

The Personal Monitor

The purpose of the personal monitor was to collect ETS particles and nicotine from the air close to the subject's breathing zone throughout a 24 hour period. The monitor consisted of an aluminium filter holder (Figure 1) which rested in the centre of the

subject's chest, supported by a rigid wire necklace. The filter holder was connected to a battery-operated pump (Model 222-3, SKC Ltd., Dorset, England) by flexible plastic tubing. The pump was concealed in a small, padded bag, attached to a belt around the subject's waist and was almost inaudible when operating within this bag.

The aluminium filter holder contained two filters in series: a Teflon front filter (FALP 02500, Millipore UK Ltd., Hertfordshire, England) and a second filter (Fiberfilm T60A20, Pallflex Corp., Connecticut, USA) acidified with sodium bisulphate. The front filter was shown to collect all ETS particles and the acidified filter was shown to retain nicotine very efficiently. The front filter was sandwiched between a stainless steel sealing ring and a stainless steel supporting grid. In prototype designs Teflon seals and spacers were used but this resulted in weight changes in the front filter of several micrograms. The use of stainless steel rings and supporting grids eliminated this weight change. The acidified filter was sandwiched between Teflon spacers and this eliminated losses of nicotine within the filter holder.

The pump flowrate was set to 139 mL min^{-1}, giving a total air sample of 200 litres in the 24-hour sampling period. At this flow rate, and with a fully-charged battery, a pump would run for about 30 hours when attached to the filter holder.

The filter holder was fitted with a security tag which ensured that the subject could not dismantle the holder without detection. Sealing caps were used to exclude air from the filter holder before and after the sampling period.

ANALYTICAL PROCEDURES

Particles from all sources

Particles from all sources were trapped on the Teflon front filter in the personal monitor. The weight collected was determined to the nearest microgram by weighing the filter before and after the monitoring period. These weighings could be carried out with good precision providing that static charge was eliminated from the filters prior to weighing. This was achieved using a commercially available, radioactive, static eliminator (PDV-1 Amersham International plc. England).

Experiments in a Model Room demonstrated that the collection efficiency for ETS particles using a personal monitor at a flow rate of 139 mL min^{-1} was equivalent to that of commercially available filter holders at the 'standard' flow rate of 2 L min^{-1}. This result is to be expected for small ($< 1\ \mu m$) particles such as those present in ETS. Calculations using the sampling rate and orifice dimensions of the personal monitor indicate that the particle collection efficiency would decrease with particle size, approaching zero efficiency at about 50 μm diameter.

Particle concentrations measured by the personal monitor do not correspond to either Total Suspended Particles (TSP) or Respirable Suspended Particles (RSP) but will fall somewhere between these values. In this publication, the term PAS is used to refer to Particles from All Sources as collected by the personal monitor. Since, in most

situations, RSP is a major fraction of TSP (Monn et al. 1993), the PAS results in this study would be expected to be similar to RSP for most subjects.

Estimation of the ETS contribution to particles from all sources

UV, fluorescence and solanesol measurements were used to estimate the ETS contribution to the total quantity of particles collected by the front filter of the personal monitor. The use of these three methods has been discussed by Ogden et al. (1990).

For the UV and fluorescence measurements, the particles were extracted from the Teflon front filter with methanol. An aliquot of the extract was injected into a column-less high performance liquid chromatography (HPLC) system and passed through a UV detector (325 nm) and a fluorescence detector (excitation 300 nm, emission 420 nm) in series. The peak areas of the UV and fluorescence signals obtained were used to calculate the quantity of ETS particles in the extract using predetermined conversion factors.

The solanesol content of the methanol extract was determined by reverse phase HPLC using methanol as the mobile phase and UV detection at 210 nm. The quantity of solanesol in the extract was converted to a quantity of ETS particles using a predetermined factor.

The factors used to convert UV, fluorescence and solanesol measurements into weights of ETS particles were established by experiments in a Model Room. ETS was generated by humans smoking combinations of typical UK cigarettes (five best-selling brands) and

ETS particles were collected from the Model Room atmosphere using the personal monitors. A range of ETS particle weights were collected on personal monitor filters by varying the sampling time. The UV absorption, fluorescence and solanesol content were measured and the relationship with weight of particles determined.

After the initial calibration against ETS, UV measurements were calibrated against a surrogate standard of 2,2',4,4'-tetrahydroxybenzophenone (THBP) and fluorescence measurements were calibrated against scopoletin as a surrogate standard. These surrogate standards were used for calibration purposes throughout the study.

The relationships obtained were reasonably consistent with those reported by Ogden et al. (1990) for US cigarettes (Table 1).

The ETS particles determined by the UV and fluorescence methods are commonly referred to as UVPM and FPM respectively. ETS particles determined by the solanesol method are referred to as SolPM in this paper.

Nicotine

Extracts of the front and back filters were basified with sodium hydroxide. Nicotine was extracted into di-isopropyl ether containing N-ethylnornicotine as an internal standard and triethylamine to prevent adsorption of nicotine by glassware. Nicotine was measured by megabore capillary GC with nitrogen-selective detection. It was shown by parallel sampling experiments that results for nicotine obtained using the personal monitor were

equivalent to those where nicotine was collected on sorbent tubes containing XAD-4 resin, as used by Ogden et al. (1989).

Saliva Cotinine

The salivette containing the saliva sample was thawed and then centrifuged to release the saliva from the cotton swab. N-Ethylnorcotinine was added to a 0.5 mL aliquot of the saliva sample as an internal standard. The aliquot was made basic with ammonium hydroxide and then cotinine and the internal standard were extracted into dichloromethane and analysed by capillary GCMS in the ion monitoring mode. Mass 176 was used to detect cotinine and mass 190 to detect the internal standard. Mass 98 and mass 112 were used as confirmatory ions for cotinine and N-ethylnorcotinine, respectively.

Mass spectrometry was used because preliminary tests had shown that nitrogen-selective detection lacked adequate selectivity at low cotinine levels. There were several nitrogen-containing compounds at similar retention times to cotinine in some saliva samples and this made it difficult to confirm the presence or absence of cotinine.

Detection limits

Detection limits for the analytical methods are listed in Table 2. For each sampling day one 'blank' personal monitor was assembled and dismantled at the same times as the subjects' personal monitors. Filters from the blank personal monitor were analysed in the same way as those from the used monitors.

Limits of detection were established by taking account of results from the blanks together with the signal to noise ratio on the real samples and the calibration standards. A signal to noise ratio of three was required for a positive signal. Results for the blank monitors were not subtracted from the results for the subjects' monitors.

The front filter used for measuring PAS was weighed as a blank throughout the study. These weighings indicate the weight change was rarely more than 1 μg. On this basis, a weight change of 4 μg was considered real and measurable for a subject's filter. This corresponds to 4 μg in the 200 litres of air collected or 20 $\mu g\ m^{-3}$ of PAS. In practice PAS results were not below this detection limit for any subject.

For many of the analyses, the levels found were below the limit of detection. This raises the question of how to deal with these results in the calculation of means, medians etc in the data analyses. If a value of zero was applied when results are below the limit of detection then this would lead to an underestimate of average exposure. Conversely, if the value of the detection limit itself was applied in such cases then the average exposure would be overestimated. As a reasonable compromise, a value which is one half of the detection limit was used for the data analysis. The same compromise has been used in other studies of this type, e.g Kirk et al. 1988, and Nehls et al. 1973.

RESULTS AND DISCUSSION

Subjects Studied

From the 327 volunteers recruited for the study, 72 were excluded for various reasons which included 7 who admitted smoking during the monitoring period. For the remaining 255 subjects, the age and sex distribution is listed in Table 3.

Excluded Subjects

Subjects and their corresponding analytical results were excluded from the study for various reasons. These are listed in Table 4.

It is debatable when using saliva cotinine levels what cut-off point should be used when attempting to distinguish between smokers and non-smokers. In a review of saliva cotinine levels, Etzel (1990) reported that non-smokers usually have levels below 5 ng mL^{-1} but that heavy exposure can result in levels around 10 ng mL^{-1}. In this study, a higher threshold of 25 ng mL^{-1} was chosen since this would avoid the possibility that some heavily exposed non-smokers might be incorrectly categorised as smokers.

The number of subjects in this study that would have been rejected as smokers at different threshold levels is given in Table 5; the seven subjects who admitted smoking are also included.

These results show that, in this study, the choice of the saliva cotinine level used as a threshold was not critical, especially in the range 15 to 30 ng mL^{-1}.

It is of interest that a further 12 subjects admitted smoking between recruitment and the 24 hour monitoring period (but not during the monitoring period itself) and yet they were not identified as smokers by their saliva cotinine measurements. This demonstrates that saliva cotinine measurements can fail to identify occasional smokers who have not smoked for a few days and could thus underestimate the extent to which smokers describe themselves as non-smokers. It is possible that further subjects in this study were occasional smokers, or smokers of very low yielding cigarettes, and were not detected.

These findings demonstrate that it can be very difficult to determine whether a person really is a regular smoker, non-smoker or even a casual smoker. This clearly needs to be taken into account in other investigations of ETS exposure.

Misclassification of Smoking Status

It was not anticipated that misclassification would occur to a significant extent in this study since the subjects were members of a well-documented group used for medical trials, and all subjects completed a recruitment questionnaire confirming their non-smoking status. In view of the recruitment criteria, it was surprising that 7 subjects admitted smoking during the 24 hour monitoring period (questionnaire), 12 subjects admitted smoking since recruitment (questionnaire), and 34 subjects were rejected on the

basis of their saliva cotinine measurement. Thus 53 subjects out of a total of 327 (ie 16%) were identified as likely smokers and some of these were clearly regular smokers.

It is conceivable that some of the 34 subjects identified as possible smokers by saliva cotinine levels were really users of nicotine gum or a nicotine patch. As misclassification was not expected to occur at a significant level in this study, the questionnaire did not address possible use by the subjects of gum or patches. However, none of these subjects mentioned the use of nicotine gum or a patch, even though questioned in detail about their smoking history. Furthermore, nicotine patches were not in common use at the time of the study.

After completion of the study, the 34 subjects were contacted by the Besselaar Clinic and asked whether they had ever used nicotine gum or a nicotine patch. From the 17 that replied 16 stated that they had used neither. This suggests that the use of nicotine gum or a nicotine patch was not likely to be a significant factor in the identification of these subjects as smokers.

The median level of saliva cotinine for self-reported smokers (or nicotine users) in the UK was reported (Lee 1987) to be 319 ng mL^{-1} for men and 311 ng mL^{-1} for women. Subjects in this study with saliva cotinines over 100 ng mL^{-1} could be regarded as regular smokers (based on an EPA definition, 1992) even though they had described themselves as non-smokers during recruitment.

The number of subjects found to have saliva cotinine levels greater than 25 ng mL^{-1} and their ranges is shown in Table 6.

Those subjects with saliva cotinine levels between 25 and 100 ng mL^{-1} may be either occasional smokers or regular smokers who had refrained from smoking just prior to the test period. The misclassified subjects on this study are listed by age and sex in Table 7.

The occupations of the misclassified subjects included, for example, housewives, a nurse, a butcher, civil servants, a cytogeneticist, a greenkeeper, clerks, labourers, a joiner, VDU operators, a laboratory technician, a musician and unemployed people.

These results indicate that people from a wide variety of occupations mis-report their smoking status, and that misclassification may be more extensive among men (33 out of 150) than women (20 out of 176) in the UK.

Relevance of 24-hour Samples

In order to assess whether the 24-hour sampling period was typical of normal ETS exposure, subjects were asked to compare their exposure during the sampling period with their average exposure over the last six months. The results are shown in Table 8.

Over 50% of subjects assessed their exposure in the monitoring period as typical of their average exposure during the last six months and less than 10% considered their exposure in the 24-hour period to be much less than normal. However, these results indicate that, on balance, the subjects judged their exposure to have been somewhat less than normal.

Objective evidence that the monitoring period was similar to recent exposure is that the mean, median and distribution of results for the pre-monitoring saliva cotinine levels are in close agreement with those of the post-monitoring values. Mean pre-cotinine and post-cotinine were both 1.4 ng mL^{-1} and the median pre and post-cotinine 0.7 and 0.6 ng mL^{-1} respectively.

The vast majority of subjects reported that wearing the personal monitor did not significantly interfere with their normal lifestyle.

Weather Conditions during the Study

The Study was conducted in October, November and early December, 1992. It was

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decided to avoid the summer months when ETS exposure was likely to be at a minimum and to avoid peak winter conditions when exposure would probably be at its highest and the practical difficulties of conducting the study at their greatest. The months chosen for the study were considered to be a good compromise.

There was a wide variation in weather conditions (as provided by the local weather centre) throughout the study. A summary is provided in Table 9.

There were three days on which hail and three days on which sleet were observed.

No obvious relationship could be seen between weather conditions and ETS exposure and there were no consistent trends when results were compared on a week-by-week basis.

LEVELS OF EXPOSURE TO ETS FOR ALL SUBJECTS

Table 10 shows the summary analytical data for all subjects.

When reporting results, both the mean and the median values of data sets are quoted together with the range of values. In this type of study where the results are far from being normally distributed the median is a more appropriate measure than the mean. This is because one or two exceptionally high values can have a disproportionately large effect on the mean when most of the other values are relatively low.

Except in the case of PAS, the median exposure values reported in this table are close to, or below, the detection limits of the methods used. This is consistent with the subjective assessments in Figure 2, where it can be seen that about 80% of subjects considered that their ETS exposure was 'none' or 'low'.

Figures 3 and 4 show the distributions of SolPM, nicotine and saliva cotinine results. The distributions are very similar in each case and show that the exposure of most subjects is at the low end of the range. Over 70% of subjects were exposed to less than 10 $\mu g\ m^{-3}$ of ETS particles, over 60% were exposed to less than 1 $\mu g\ m^{-3}$ of nicotine and over 60% had saliva cotinine less than 1 ng mL^{-1}.

Results for the 10% of subjects with the highest measured ETS exposure, based on SolPM, are also shown in Table 10.

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In Table 10, the differences between the values for UVPM, FPM, and SolPM reflect the susceptibility of these measurements to interference. In some cases, FPM and especially UVPM, were observed at fairly high levels when there was no evidence of smoke exposure, clearly demonstrating the presence of interference. The solanesol-based measurements were more consistent with nicotine measurements and reported ETS exposure. For these reasons, discussion of ETS particle values in this paper relate to those estimated by the SolPM method.

Nicotine levels found in this study are in good agreement with those reported in other recent studies, including a study by Proctor et al. (1991) in which the nicotine exposure of women in Birmingham, England was measured by a personal monitoring technique. The saliva cotinine results are also very similar to those reported by Proctor et al. (1991). Table 11 compares these findings.

Ogden et al. (1993), in a personal monitoring study of non-smoking women in the USA, reported median nicotine exposure of 0.43 $\mu g\ m^{-3}$ and median saliva cotinine of 0.8 $ng\ mL^{-1}$.

ETS particles were found to make only a small contribution to PAS (Table 12).

Assuming a breathing rate of 1 m^3 per hour, a person exposed to the median levels found in this study would be exposed to about 1,244 mg of PAS, 17.5 mg of ETS particles and about 4.4 mg of nicotine in a year. This calculation assumes these subjects are exposed at this 24 hour median level throughout the year.

Based on the median levels for the tenth decile (SolPM), the most heavily exposed subjects would be exposed to approximately 1944 mg PAS, 561 mg of ETS particles. and 63 mg nicotine in a year. This calculation assumes these subjects are exposed at this higher 24 hour level throughout the year.

For comparison, a typical UK cigarette delivers about 12 mg of particles and 1 mg of nicotine to the smoker.

By modelling of fixed-site RSP measurements, Repace and Lowrey (1993) estimated typical daily non-smoker exposure in the USA, corresponding to 522 mg/year for ETS particles and 52 mg/year for nicotine. For the "most-exposed" non-smokers. their estimates correspond to an ETS particle exposure of 5220 mg/year and a nicotine exposure of 522 mg/year. The data reported here suggest that non-smoker exposure to ETS in the UK is well below that estimated by Repace and Lowrey for the USA.

Summary analytical results by age and sex are given in Tables 13 and 14. Male subjects were found to have higher average exposure to ETS particles and nicotine. and had higher saliva cotinine levels. The highest exposure for males and females was in the 21 to 29 age group.

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ETS EXPOSURE FROM WORK, HOME, LEISURE AND TRAVEL

Questionnaire data indicate that, on average, relative contributions to total ETS were subjectively ranked as Leisure > Work > Home > Travel, both during the monitoring period and during the last six months (Table 15).

Subjects with a non-smoking spouse/partner reported their primary source of ETS exposure as Leisure (48% of cases), Work (34% of cases), and Travel (3% of cases). However subjects with a smoking spouse/partner reported their main source of exposure as Leisure (19% of cases), Work (11% of cases), and Travel (0% of cases). The spouse was assessed as the primary source of exposure by 53% of these subjects.

Since subjects were not separately monitored at home, work, leisure or during travel it is not straightforward to assess the contribution of each of these sources to the 24 hour exposure. It is necessary to rely on information from the subjects about where they were exposed.

Most subjects either assessed their ETS exposure as 'none' or reported that their exposure was from more than one source. However, in those cases where the 24-hour result was attributed by the subject to a single source, the measured exposure levels, based on the means, suggest that the ranking is Home > Leisure > Work > Travel (Table 16).

In view of the small numbers of subjects having a single source of exposure, another approach was used to assess the home, work, leisure and travel contributions to total ETS

exposure. For each subject exposed to more than $10 \mu g\ m^{-3}$ of ETS particles, the assessment of percentage exposure from the four sources was used to distribute the corresponding measured 24-hour values between the four sources. Where the subjects assessed their 24 hour exposure as 'none', the corresponding measured values were distributed according to the number of hours the subject spent at each source.

The mean results computed using this method again suggest that the ranking is Home > Leisure > Work > Travel. These computed estimates are shown in Table 17.

The subjective and measured results both suggest that, overall, travel makes only a minor contribution to exposure.

It is interesting that the ranking of sources of exposure found by measurement is different from the ranking perceived by the subjects. One possible explanation for this is that subjects have based their judgements on the relative ETS <u>levels</u> in the four situations while taking insufficient account of the time spent in these situations.

SPOUSAL SMOKING

In order to examine the effect of spousal smoking on ETS exposure, the subjects' results were divided into 3 groups depicted in Table 18.

Figure 5 shows the subjective assessments of ETS exposure made by the three groups. These subjective results indicate that the ranking of exposure is Smoking partner > No partner > Non-smoking partner, although there is considerable overlap between individual subjects within the groups. In the group with a smoking spouse/partner: 53% assessed their spouse/partner as their primary source of ETS exposure, 30% assessed work or leisure as their primary source of exposure, and 16% of subjects assessed their exposure as 'none'.

The measured exposure levels and the saliva cotinine data for these groups (Table 19) suggest the same overall ranking as the subjective data. The distribution of results for these groups (see Figure 6 for the distribution of ETS particles results) confirms that there is considerable overlap between the amount of exposure of individuals in these groups. Furthermore, 29% of subjects with a smoking partner were exposed to less than the mean ETS particle level of subjects with a non-smoking partner.

Figure 6 shows that 40% of subjects with a smoking spouse/partner have exposure in the lowest SolPM range. This is in agreement with the subjective exposure assessments of 'none' and 'low' chosen by 45% of subjects in this group.

These findings indicate that a group of subjects with a smoking spouse/partner is likely to have a higher average ETS exposure than a group with a non-smoking spouse/partner. However, spousal smoking status clearly cannot be used, without supplementary evidence, to assess reliably the ETS exposure of individuals or small groups of subjects.

It can be seen from the median values in Table 19 that subjects with a smoking partner were exposed to about nine times more ETS particles and nicotine than subjects with a non-smoking partner. Corresponding data for women (Table 20) shows that the ETS particle exposure for those with a smoking partner was 5.5 times greater than for women with a non-smoking partner. The ratio was 6.5 for nicotine exposure and an average of 4.4 based on saliva cotinine. These ratios are higher than the ratio of 1.75 assumed for women in the USA by the EPA (1992) in their ETS risk assessment. A recent study carried out in the USA (Ogden et al. 1993) has also found these exposure ratios to be higher than 1.75.

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COMPARISON OF OTHER MEASURES OF SMOKE EXPOSURE

Subjective Assessments Compared with Direct Measurements

Figures 7 and 8 show the variation in measured exposure and saliva cotinine level for each grade of subjective exposure assessment. Although subjects were able to assess an exposure of 'none' or 'low' quite well, there was considerable variation in the direct measurements corresponding to the higher grades of subjective assessment. Some subjects who reported their exposure as 'high' had less directly measured exposure than other subjects who reported their exposure as 'low'.

Clearly, therefore, an individual's ETS exposure cannot be reliably assessed by a simple questionnaire. Better assessments may be possible by detailed questioning about the hours spent in various locations and the numbers of smokers present, but this was not examined in this study. Delfino et al. (1993) did investigate this issue and concluded that "the weak relationship between questionnaire estimates of ETS exposure and cotinine found in the present study, suggests that further investigation is needed to improve the assessment of recent ETS exposure".

Saliva Cotinine Compared with Direct Measurements

Saliva cotinine values showed poor correlation ($R^2 = 0.06$ for pre, $R^2 = 0.14$ for post) with 24-hour ETS particle exposure and, perhaps surprisingly, poor correlation with 24-hour nicotine exposure ($R^2 = 0.07$ for pre, $R^2 = 0.13$ for post). Some subjects exposed

to relatively high levels of ETS particles and nicotine had no detectable saliva cotinine. Conversely, some subjects who had not been exposed to any measurable quantity of nicotine had relatively high levels of saliva cotinine. Possible reasons contributing to the lack of correlation could be:

(1) Different rates of nicotine metabolism between subjects.

(2) For many subjects, the saliva cotinine value was close to, or below, the limit of detection.

(3) ETS exposure occurring at different times within the 24-hour period for different subjects.

(4) The previous days' exposure determining the pre-cotinine level and the residual pre-cotinine contributing to the post-cotinine level.

(5) Dietary consumption of nicotine.

Poor correlations of nicotine exposure with saliva cotinine levels have also been found in other recent studies (Proctor et al. 1991) and therefore the value of saliva cotinine measurements for estimation of nicotine exposure at low levels must be seriously questioned.

Comparison of Methods for Estimating ETS Particles

Three methods were used in this study for estimating the ETS contribution to particles from all sources and it might be expected that there would be a good correlation between the results obtained. The relatively poor correlation between UVPM and

SolPM (R^2=0.19) and the moderate correlation between FPM and SolPM (R^2=0.46) can be largely explained by interference in the UVPM and FPM measurements. In some cases where SolPM and nicotine are very low, i.e there was very little ETS exposure, there were relatively high levels of UVPM or FPM (Figure 9). Figure 10 shows that if these most obvious cases of interference were removed, there would be an improvement in the correlation between UVPM and SolPM and between FPM and SolPM. Also, the slopes of the lines of best fit would then approach, more closely, the expected value of 1.0.

Comparison of Nicotine and ETS Particle Exposures

ETS particles and nicotine are generated in a reasonably consistent ratio during smoking. However, nicotine is predominantly in the gas phase in ETS and is known to diffuse and decay more rapidly than ETS particles (Eatough et al. 1987). Furthermore, nicotine absorbs on walls, fabrics etc, and can be subsequently released into an atmosphere after ETS particles have disappeared. Therefore, at a given time, the ratio of nicotine to ETS particles may be quite different from the ratio in which they were generated (Nelson et al. 1992). However, in this study, a reasonably good correlation (R^2 = 0.66) was found between levels of nicotine and ETS particles. This was possibly because the sampling period was relatively long, thereby smoothing out shorter term fluctuations in the nicotine to ETS particles ratio.

Comparison of Exposure to ETS Particles with Exposure to Particles from All Sources

There is no obvious reason why exposure to ETS particles should correlate with exposure to particles from other sources, and the total lack of correlation ($R^2 = 0.04$) found in this study is, therefore, not surprising. Clearly, measurements of particles from all sources, or Respirable Suspended Particles (RSP), should not be used to assess ETS exposure and it is essential to estimate the contribution ETS makes to total particles.

CONCLUSIONS

The results of this study indicate that, for most subjects, exposure to nicotine and ETS particles was close to, or below, the limits of detection of the methods used. These low exposure levels are consistent with the corresponding assessments of exposure made by the subjects, and their saliva cotinine levels. The results are also consistent with other recent studies of ETS exposure by personal monitoring.

At the median level of ETS found in this study, a person would be exposed to approximately 1,244 mg of particles from all sources, 17.5 mg of ETS particles and 4.4 mg of nicotine in a year, assuming they were exposed at this 24 hour level throughout the year.

Based on the median levels for the tenth decile (SolPM), the most heavily exposed subjects would be exposed to approximately 1944 mg PAS, 561 mg of ETS particles, and 63 mg nicotine in a year. This calculation assumes these subjects are exposed at this higher 24 hour level throughout the year.

For comparison, a typical UK cigarette delivers about 12 mg of particles and 1 mg of nicotine to the smoker.

The above estimates of annual exposure to ETS are well below those obtained by Repace and Lowrey using a model based on fixed-site RSP measurements in the USA.

Although subjects' assessments indicated that Leisure was the major source of ETS exposure, measurements by personal monitoring showed that, on average, the Home was the most important, followed by Leisure and Work. Travel was only a minor contributor to ETS exposure.

Subjects' assessments of their ETS exposure were not a reliable guide to their measured exposure except when their assessment was 'none' or 'low'. Individuals varied considerably in what they considered to be 'moderate' or 'high' levels of ETS exposure.

On average, subjects with a partner who smokes had about nine times greater exposure to nicotine and ETS particles than those with no partner or a non-smoking partner. However, there was considerable overlap in the exposure of individuals between these groups. For example, 29% of subjects with a smoking partner were exposed to less than the mean ETS particle level of subjects with a non-smoking partner. Clearly, spousal smoking status would not be a reliable means to assess the ETS exposure of individuals or small groups of subjects.

There was a moderate correlation between exposure to nicotine and exposure to ETS particles as measured by solanesol (SolPM). As might be expected, there was a very poor correlation between these components of ETS and exposure to particles from all sources. Overall, ETS made a small contribution (median 2.5%) to particle exposure and it is clear that exposure to particles from all sources cannot be used as a reliable measure of ETS exposure.

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There was a surprisingly poor correlation between ETS nicotine exposure and saliva cotinine level. However, other recent studies have drawn the same conclusion and further work should be undertaken to establish the reliability of saliva cotinine measurements as a means of assessing ETS exposure.

There was a greater than expected number of subjects (41) who had to be eliminated from the study for suspected smoking even though they were recruited as non-smokers. A saliva cotinine threshold of 25 ng mL^{-1} was used to distinguish between smokers and non-smokers. Results show that the threshold value used is not very critical, especially in the range 15 to 30 ng mL^{-1}.

Saliva cotinine measurements failed to identify a further 12 subjects who admitted occasional smoking since recruitment. It is, therefore, possible that other subjects who did not admit occasional smoking were also not identified. In total 53 subjects from an original 327 volunteers were identified as having smoked since recruitment, corresponding to a 16% misclassification rate. The rate was also significantly higher among men (22%) than women (11%).

Overall, this study has shown that it is feasible to assess exposure to nicotine and ETS particles by personal monitoring. This method of assessing ETS exposure would be expected to be more accurate than the saliva cotinine and questionnaire/lifestyle approaches that are sometimes used.

Since the ETS exposure for most subjects was low and close to the detection limits of the methods used in this study, it would be advisable to improve the precision of measurements at the lowest levels by using a longer monitoring period in future studies.

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Table 1

A COMPARISON OF THE QUANTITIES OF ETS PARTICLES WHICH CORRESPOND TO 1 μg OF EACH SURROGATE STANDARD AS DETERMINED IN THIS STUDY AND IN A STUDY BY OGDEN ET AL

	OGDEN et al.	This Study
1 μg THBP (used for UVPM)	8.0 μg ETS particles	6.1 μg ETS particles
1 μg scopoletin (used for FPM)	33.6 μg ETS particles	22.5 μg ETS particles
1 μg solanesol (used for SolPM)	30.0 μg ETS particles	38.5 μg ETS particles

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Table 2

DETECTION LIMITS OF THE ANALYTICAL METHODS USED

PAS	20 $\mu g\ m^{-3}$
UVPM	8 $\mu g\ m^{-3}$
FPM	4 $\mu g\ m^{-3}$
SolPM	4 $\mu g\ m^{-3}$
Nicotine	0.1 $\mu g\ m^{-3}$
Saliva cotinine	0.5 $ng\ mL^{-1}$

PAS = particles from all sources

UVPM = ETS particles measured by UV

FPM = ETS particles measured by Fluorescence

SolPM = ETS particles measured by Solanesol

Table 3

AGE AND SEX DISTRIBUTION FOR STUDY SUBJECTS

Age range (Years)	Males	Females	Total
21 to 29	54	57	111
30 to 39	26	37	63
40 to 49	16	20	36
50 to 61	12	33	45
Total	108	147	255

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Table 4

EXCLUSION CRITERIA FOR STUDY SUBJECTS

Reason	Number
Subjects did not keep their appointment	5
The personal monitor pump failed to run for the full 24 hours	6
The analytical protocol was not properly followed	14
Smoke was deliberately blown into the monitor (Questionnaire)	6
Subjects admitted smoking during the 24 hour monitoring period (Questionnaire)	7
Subjects had a saliva cotinine level above the threshold for non-smokers	34
Total Excluded	72

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Table 5

NUMBERS OF SUBJECTS CLASSIFIED AS SMOKERS AS A FUNCTION OF SALIVA COTININE REJECTION THRESHOLD

Cut Off Level ($ng\ mL^{-1}$)	Subjects that would be rejected
10 (Etzel, 1990)	47
15 (McNeill, 1987)	41
25	41
30 (L ee, 1987)	37
50	34
100	22

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Table 6

DISTRIBUTION OF SALIVA COTININE LEVELS ABOVE 25ng mL^{-1}

Number of Subjects	Cotinine Range ($ng\ mL^{-1}$)
19	25 - 100
2	100 - 150
3	150 - 200
1	200 - 300
7	300 - 400
7	400 - 500
2	500 - 600

Table 7

AGE AND SEX DISTRIBUTION OF MISCLASSIFIED SUBJECTS

Age range (Years)	Males	Females	Total
21 to 29	21	11	32
30 to 39	6	6	12
40 to 49	5	2	7
50 to 61	1	1	2
Total	33	20	53

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Table 8

A SUBJECTIVE COMPARISON OF ETS EXPOSURE IN THE MONITORING PERIOD AND IN THE 6 MONTHS PRIOR TO MONITORING

Subjective Assessment	Number of Subjects	% of Total
Much less than normal	25	9.8
Less than normal	91	35.7
Fairly typical of average exposure	131	51.4
More than normal	7	2.7
Much more than normal	1	0.4
Totals	255	100.0

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Table 9

WEATHER CONDITIONS DURING THE STUDY

Maximum Temperature	16.9°C	
Minimum Temperature	-3.2°C	
Maximum Humidity	96%	
Minimum Humidity	55%	
Maximum Daily Rainfall	10.4mm	
Maximum Daily Sunshine	7.5 hours	
Minimum Windspeed	2.1 ms^{-1}	(4 knots)
Maximum Windspeed	13.4 ms^{-1}	(26 knots)

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Table 10

SUMMARY STATISTICS FOR ALL ANALYTES AND ALL SUBJECTS

		Minimum	Maximum	Mean	Median	Number
PAS	($\mu g\ m^{-3}$)	20	1219	179	142	255
UVPM	($\mu g\ m^{-3}$)	4	299	31	21	255
FPM	($\mu g\ m^{-3}$)	2	146	16	10	255
SolPM	($\mu g\ m^{-3}$)	2	159	12	2	255
Nicotine	($\mu g\ m^{-3}$)	0.05	26	1.7	0.50	249
Pre-cotinine	($ng\ mL^{-1}$)	0.25	14	1.4	0.70	254
Post-cotinine	($ng\ mL^{-1}$)	0.25	12	1.4	0.60	248

SUMMARY STATISTICS FOR SUBJECTS WITH UPPER DECILE OF ETS EXPOSURE

		Minimum	Maximum	Mean	Median	Number
PAS	($\mu g\ m^{-3}$)	89	420	228	222	25
SolPM	($\mu g\ m^{-3}$)	42	159	72	64	25
Nicotine	($\mu g\ m^{-3}$)	0.44	26	8.4	7.2	25
Pre-cotinine	($ng\ mL^{-1}$)	0.25	8.4	2.3	1.5	25
Post-cotinine	($ng\ mL^{-1}$)	0.25	12	3.3	2.4	25

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Table 11

COMPARISON OF NICOTINE AND SALIVA COTININE LEVELS

FOUND IN THIS STUDY WITH ANOTHER RECENT STUDY

Nicotine ($\mu g\ m^{-3}$)		Minimum	Maximum	Mean	Median
This Study		0.05	26	1.7	0.5
Proctor et al. (1991)		0	45	2.3	0
Saliva Cotinine ($ng\ mL^{-1}$)					
This Study	Pre Sample	0.25	14	1.4	0.7
This Study	Post Sample	0.25	12	1.4	0.6
Proctor et al. (1991)	Pre Sample	0.30	15	1.8	1.2
Proctor et al. (1991)	Post Sample	0	9	1.5	1.1

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Table 12

ETS PARTICLES (SolPM) AS A PERCENTAGE OF PARTICLES FROM ALL SOURCES (PAS)

Minimum	0.2
Maximum	60.0
Mean	7.1
Median	2.5

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Table 13

SUMMARY STATISTICS OF DIRECTLY MEASURED ANALYTES FOR ALL SUBJECTS BY AGE AND SEX

Age range	Sex	Subjects	Minimum	Maximum	Mean	Median
				Nicotine ($\mu g\ m^{-3}$)		
All ages	Both	255	0.05	26	1.7	0.50
	M	108	0.05	26	2.2	0.87
	F	147	0.05	19	1.4	0.34
21-29	M	54	0.05	13	2.6	1.62
	F	57	0.05	15	1.5	0.47
30-39	M	26	0.05	26	2.0	0.40
	F	37	0.05	6	1.3	0.23
40-49	M	16	0.05	11	1.6	0.33
	F	20	0.05	19	1.6	0.31
50-61	M	12	0.05	9	1.9	0.69
	F	33	0.05	18	1.2	0.31
				Pre-Cotinine ($ng\ mL^{-1}$)		
All ages	Both	255	0.25	14	1.4	0.7
	M	108	0.25	14	1.9	1.0
	F	147	0.25	13	1.0	0.6
21-29	M	54	0.25	14	2.3	1.4
	F	57	0.25	8.2	1.1	0.7
30-39	M	26	0.25	13	2.0	0.9
	F	37	0.25	13	1.4	0.3
40-49	M	16	0.25	3.4	0.9	0.5
	F	20	0.25	2.6	0.7	0.7
50-61	M	12	0.25	4.4	1.0	0.4
	F	33	0.25	4.2	0.7	0.3
				Post-Cotinine ($ng\ mL^{-1}$)		
All ages	Both	255	0.25	12	1.4	0.6
	M	108	0.25	12	2.0	0.7
	F	147	0.25	8.1	1.0	0.25
21-29	M	54	0.25	12	2.3	1.3
	F	57	0.25	7.5	1.2	0.7
30-39	M	26	0.25	12	2.4	0.8
	F	37	0.25	7.0	0.9	0.25
40-49	M	16	0.25	7.8	1.4	0.25
	F	20	0.25	2.4	0.7	0.25
50-61	M	12	0.25	2.8	0.8	0.4
	F	33	0.25	8.1	0.9	0.25

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Table 14

SUMMARY STATISTICS OF DIRECTLY MEASURED

ANALYTES FOR ALL SUBJECTS BY AGE AND SEX

Age range	Sex	Subjects	Minimum	Maximum	Mean	Median
				PAS ($\mu g\ m^{-3}$)		
All ages	Both	255	20	1219	179	142
	M	108	20	1219	169	136
	F	147	30	847	187	151
21-29	M	54	45	995	163	129
	F	57	35	420	171	150
30-39	M	26	48	1219	177	126
	F	37	52	539	208	190
40-49	M	16	20	524	181	152
	F	20	45	847	205	144
50-61	M	12	29	286	158	155
	F	33	30	549	183	131
				SolPM ($\mu g\ m^{-3}$)		
All ages	Both	255	2	159	12	2
	M	108	2	159	15	4
	F	147	2	153	11	2
21-29	M	54	2	97	17	6
	F	57	2	153	15	2
30-39	M	26	2	159	15	2
	F	37	2	88	10	2
40-49	M	16	2	51	9	2
	F	20	2	78	9	2
50-61	M	12	2	50	13	7
	F	33	2	87	5	2

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Table 15

SUBJECTIVE ASSESSMENT OF RELATIVE CONTRIBUTIONS TO ETS EXPOSURE DURING THE MONITORING PERIOD AND THE PREVIOUS 6 MONTHS

Percent Relative Contributions

	Monitoring Period	Last Six Months
Home	27.8	19.8
Work	31.5	29.4
Leisure	35.1	46.3
Travel	5.7	4.7

Table 17

"COMPUTED" ESTIMATES OF EXPOSURE AT HOME, WORK, LEISURE AND TRAVEL

FOR ALL SUBJECTS WITH TOTAL SolPM > 10 $\mu g\ m^{-3}$

	Minimum	Maximum	Mean	Median	Number
		SolPM ($\mu g\ m^{-3}$)			
Home	0	107	18	7.2	67
Work	0	79	8.0	0	67
Leisure	0	151	12	0.2	67
Travel	0	11	1.2	0	67
Overall	10	159	39	26	67
		Nicotine ($\mu g\ m^{-3}$)			
Home	0	16	2.1	0.30	66
Work	0	9.7	1.0	0	66
Leisure	0	24	1.6	0.03	66
Travel	0	2.1	0.1	0	66
Overall	0.05	26	4.9	3.6	66

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Table 18

DISTRIBUTION OF SUBJECTS BY SMOKING STATUS OF SPOUSE OR PARTNER

	Number in Group
Subjects with no spouse or partner	74
Subjects with a non-smoking spouse or partner	133
Subjects with a smoking spouse or partner	48
Total all Categories	255

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Table 19

SUMMARY EXPOSURE MEASUREMENTS FOR ALL SUBJECTS BY CLASSIFICATION OF SPOUSE OR PARTNER

	Minimum	Maximum	Mean	Median	Number
		PAS ($\mu g\ m^{-3}$)			
NS Partner	20	995	166	129	133
SM Partner	48	1219	219	161	48
No Partner	35	539	178	143	74
Overall	20	1219	179	142	255
		SolPM ($\mu g\ m^{-3}$)			
NS Partner	2	159	7	2	133
SM Partner	2	153	29	17	48
No Partner	2	97	12	2	74
Overall	2	159	12	2	255
		Nicotine ($\mu g\ m^{-3}$)			
NS Partner	0.05	26	1.1	0.28	130
SM Partner	0.05	18	4.0	2.5	47
No Partner	0.05	19	1.5	0.55	72
Overall	0.05	26	1.7	0.5	249
		Pre-Cotinine ($ng\ mL^{-1}$)			
NS Partner	0.25	8.2	0.83	0.25	132
SM Partner	0.25	13	2.3	1.4	48
No Partner	0.25	14	1.8	1.0	74
Overall	0.25	14	1.4	0.7	254
		Post-Cotinine ($ng\ mL^{-1}$)			
NS Partner	0.25	12	0.99	0.25	128
SM Partner	0.25	8.1	2.2	1.5	47
No Partner	0.25	12	1.7	0.6	73
Overall	0.25	12	1.4	0.6	248

NS = non-smoking spouse or partner

SM = smoking spouse or partner

Table 20

SUMMARY EXPOSURE MEASUREMENTS FOR WOMEN BY CLASSIFICATION OF SPOUSE OR PARTNER

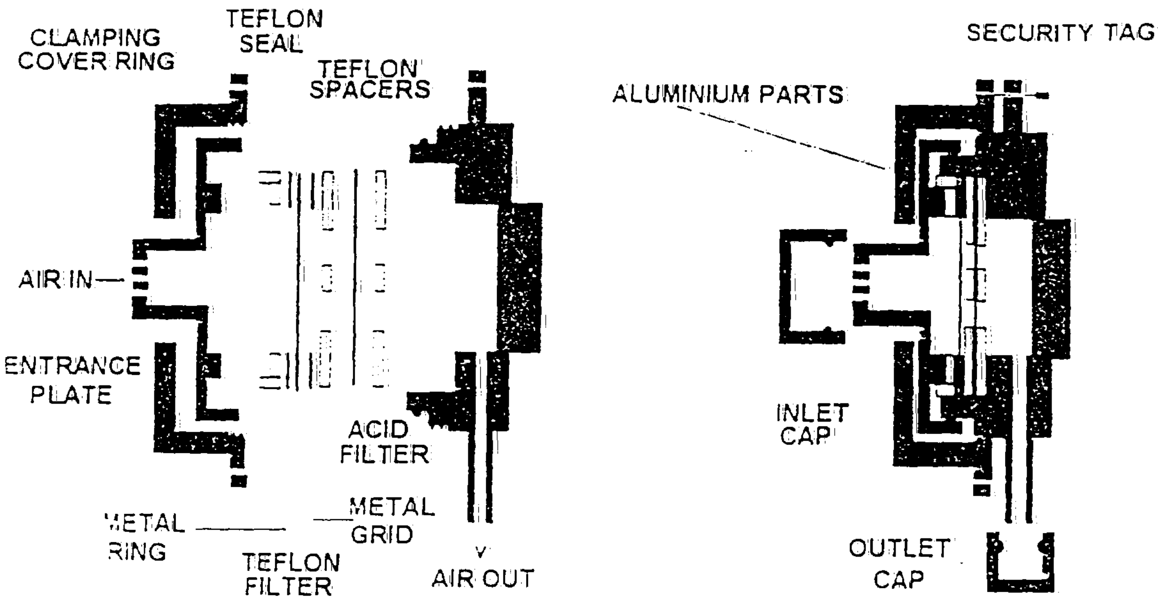
	Minimum	Maximum	Mean	Median	Number
PAS ($\mu g\ m^{-3}$)					
NS Partner	30	847	175	128	80
SM Partner	89	497	219	166	26
No Partner	35	539	192	153	41
SolPM ($\mu g\ m^{-3}$)					
NS Partner	2	87	5	2	80
SM Partner	2	153	28	11	26
No Partner	2	88	10	2	41
Nicotine ($\mu g\ m^{-3}$)					
NS Partner	0.05	11	0.81	0.23	78
SM Partner	0.05	19	3.4	1.5	26
No Partner	0.05	19	1.3	0.35	40
Pre-Cotinine ($ng\ mL^{-1}$)					
NS Partner	0.25	8.2	0.79	0.25	80
SM Partner	0.25	13	1.8	1.3	26
No Partner	0.25	8.4	1.0	0.70	41
Post-Cotinine ($ng\ mL^{-1}$)					
NS Partner	0.25	7.5	0.93	0.25	76
SM Partner	0.25	8.1	1.3	0.90	25
No Partner	0.25	7.0	0.92	0.25	41

NS = non-smoking spouse or partner

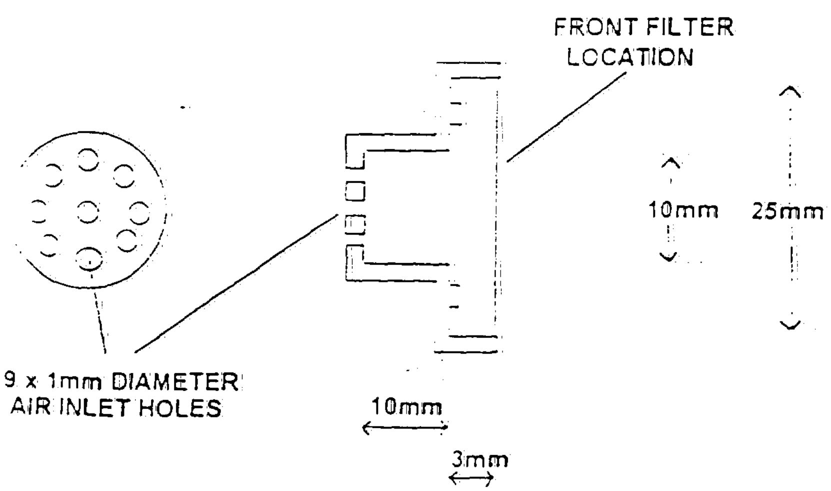
SM = smoking spouse or partner

Figure 1

THE PERSONAL MONITOR FILTER HOLDER



FILTER HOLDER APART AND ASSEMBLED



ENTRANCE PLATE DIMENSIONS

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Figure 2

SUBJECTIVE ASSESSMENT OF 24 HOUR ETS EXPOSURE

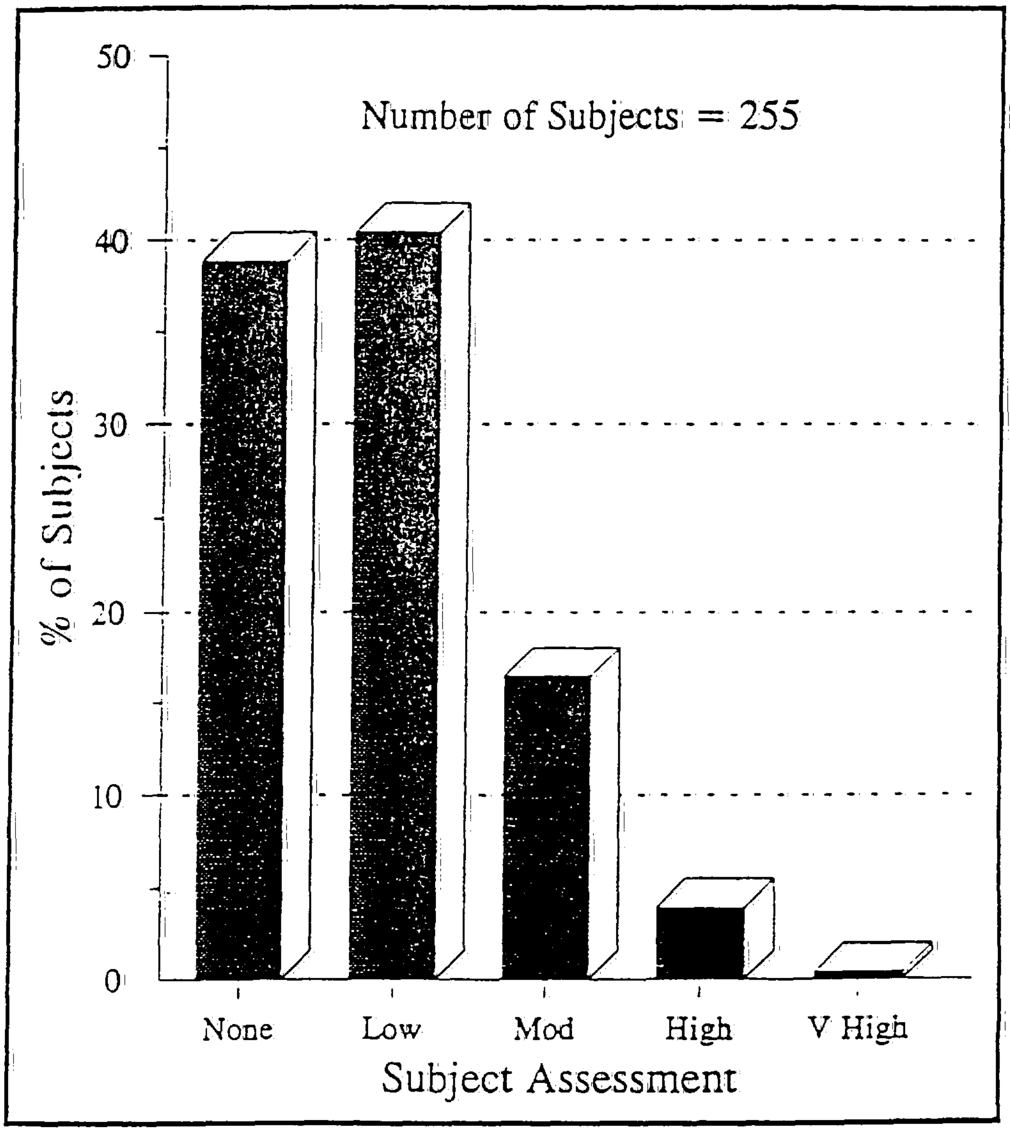

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Figure 3

DISTRIBUTION OF SolPM and NICOTINE RESULTS

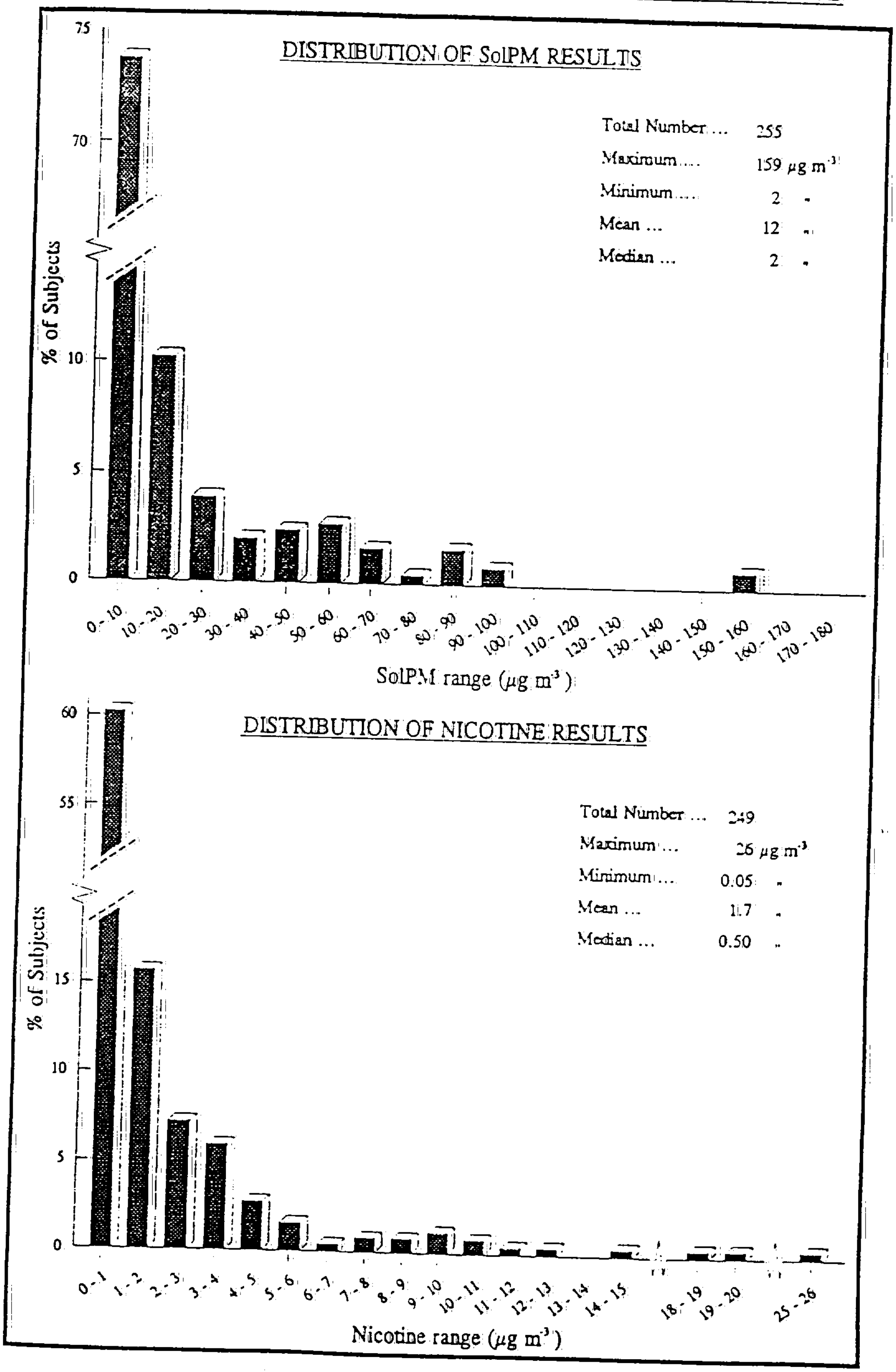

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Figure 4

DISTRIBUTION OF COTININE RESULTS

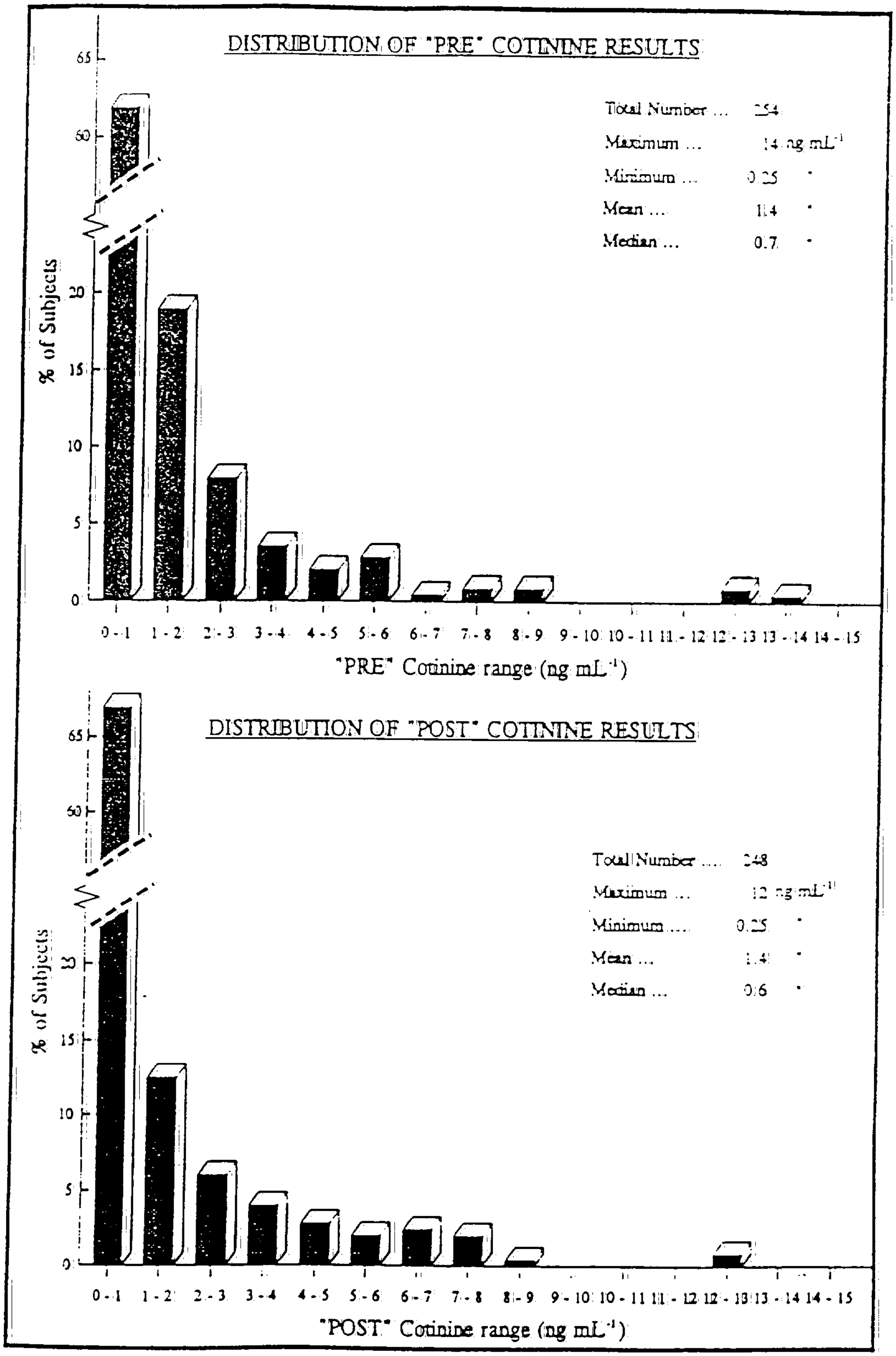

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Figure 5

SUBJECTIVE ASSESSMENT OF 24 HOUR ETS EXPOSURE BY CLASSIFICATION OF SPOUSE OR PARTNER

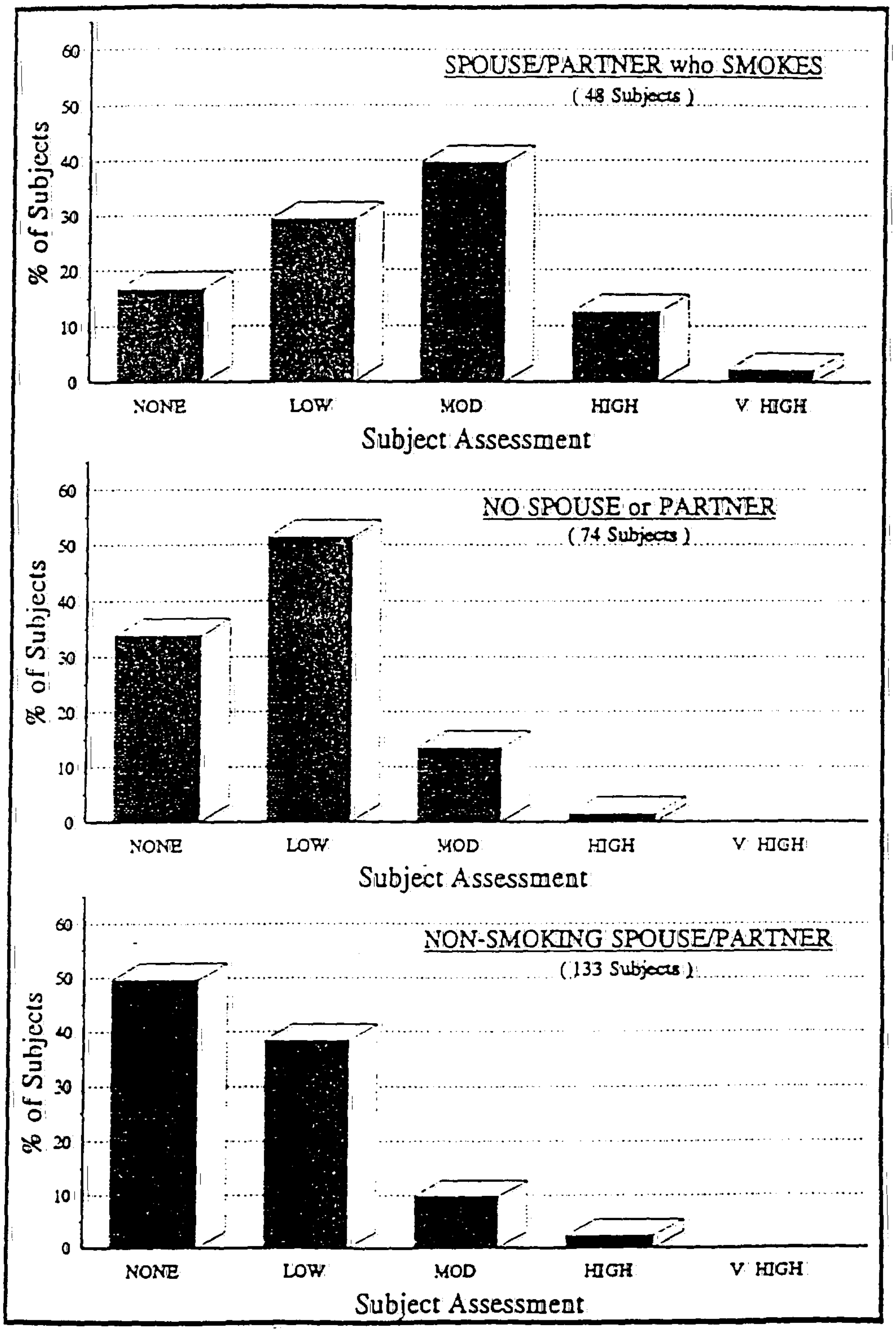

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Figure 7

COMPARISON OF SolPM and NICOTINE RESULTS WITH SUBJECTIVE ASSESSMENT OF ETS EXPOSURE

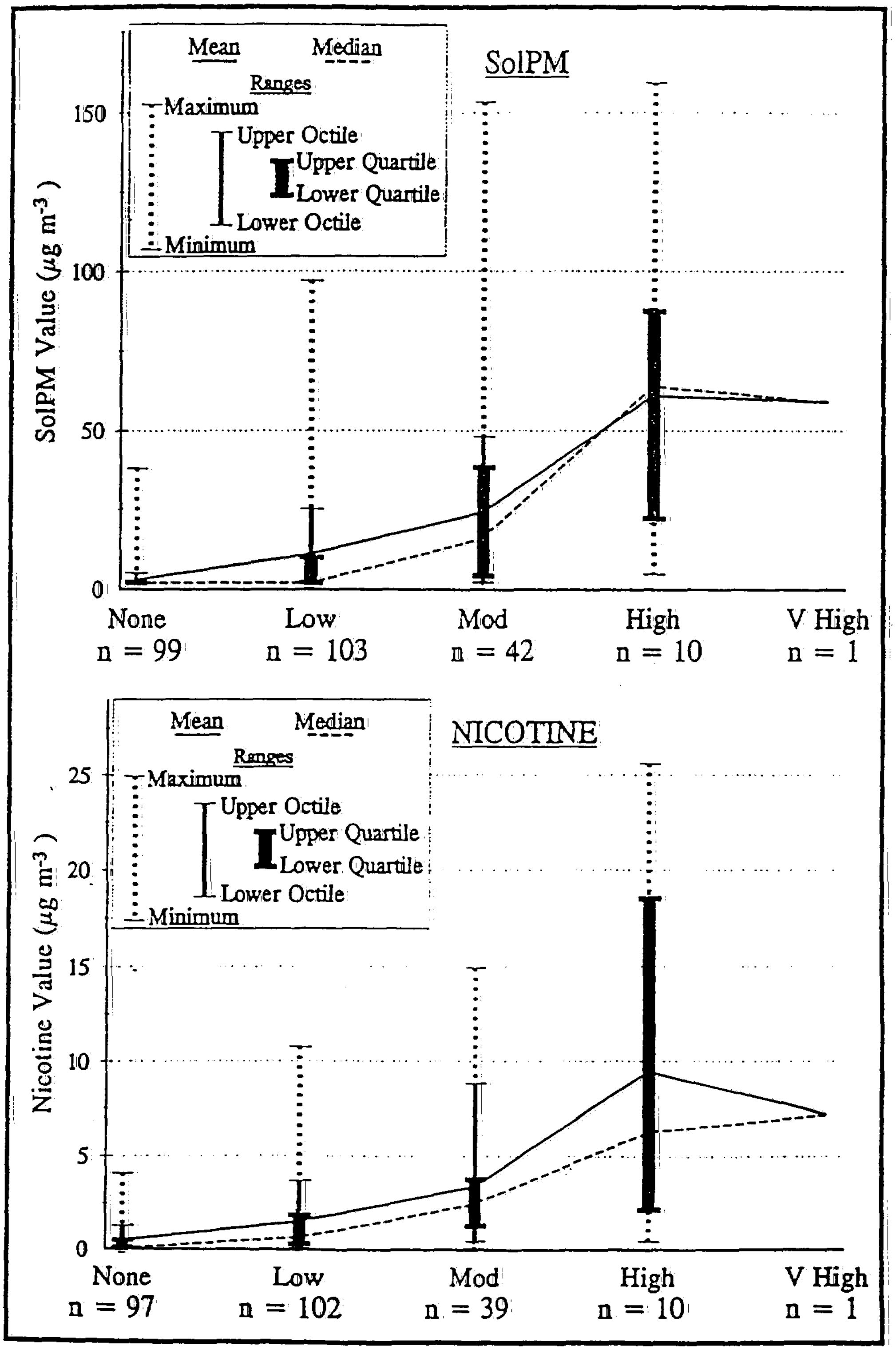

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Figure 8

COMPARISON OF COTININE RESULTS WITH SUBJECTIVE ASSESSMENT OF ETS EXPOSURE

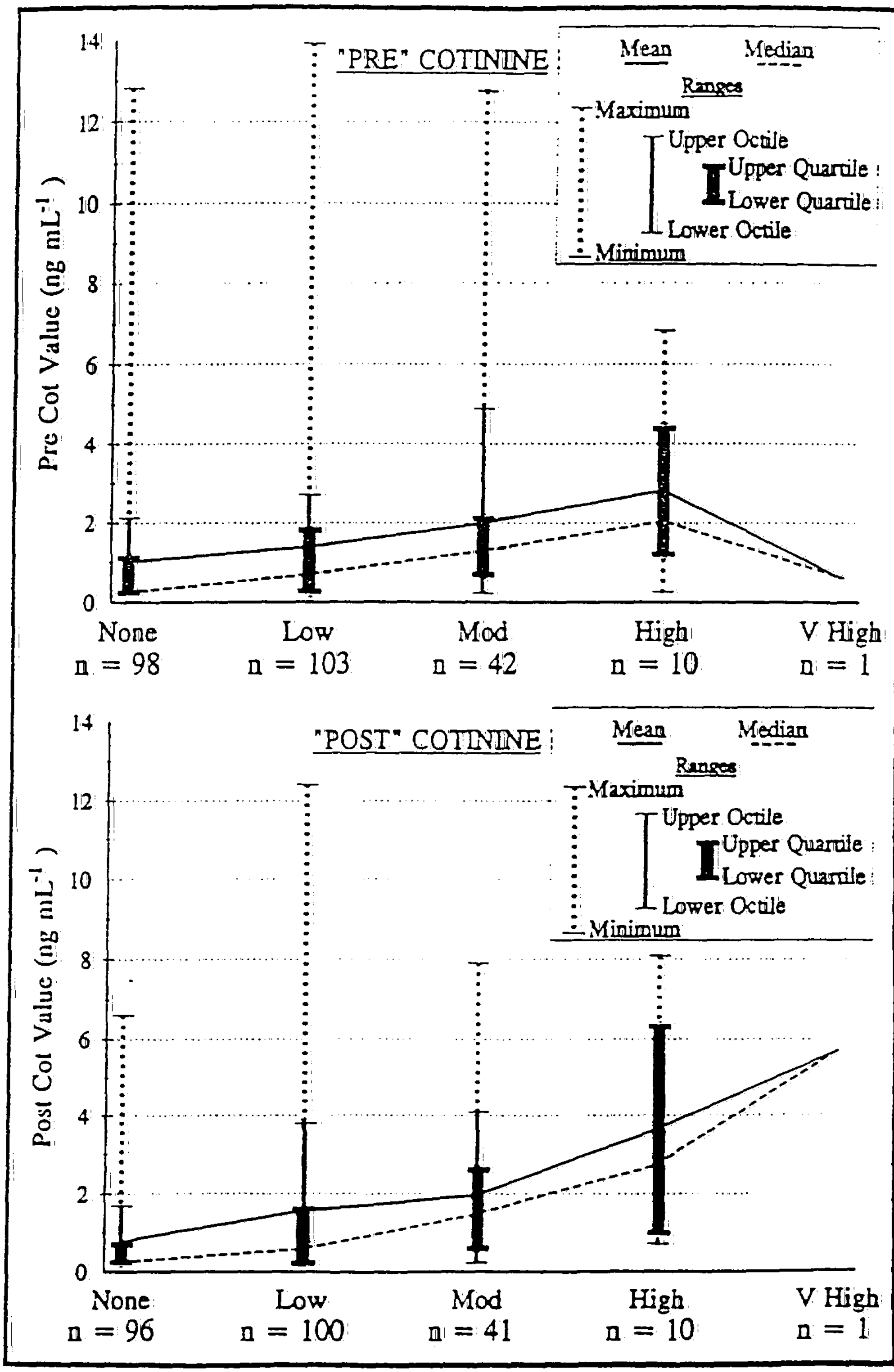

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Figure 9

CORRELATION OF SolPM RESULTS WITH UVPM and FPM

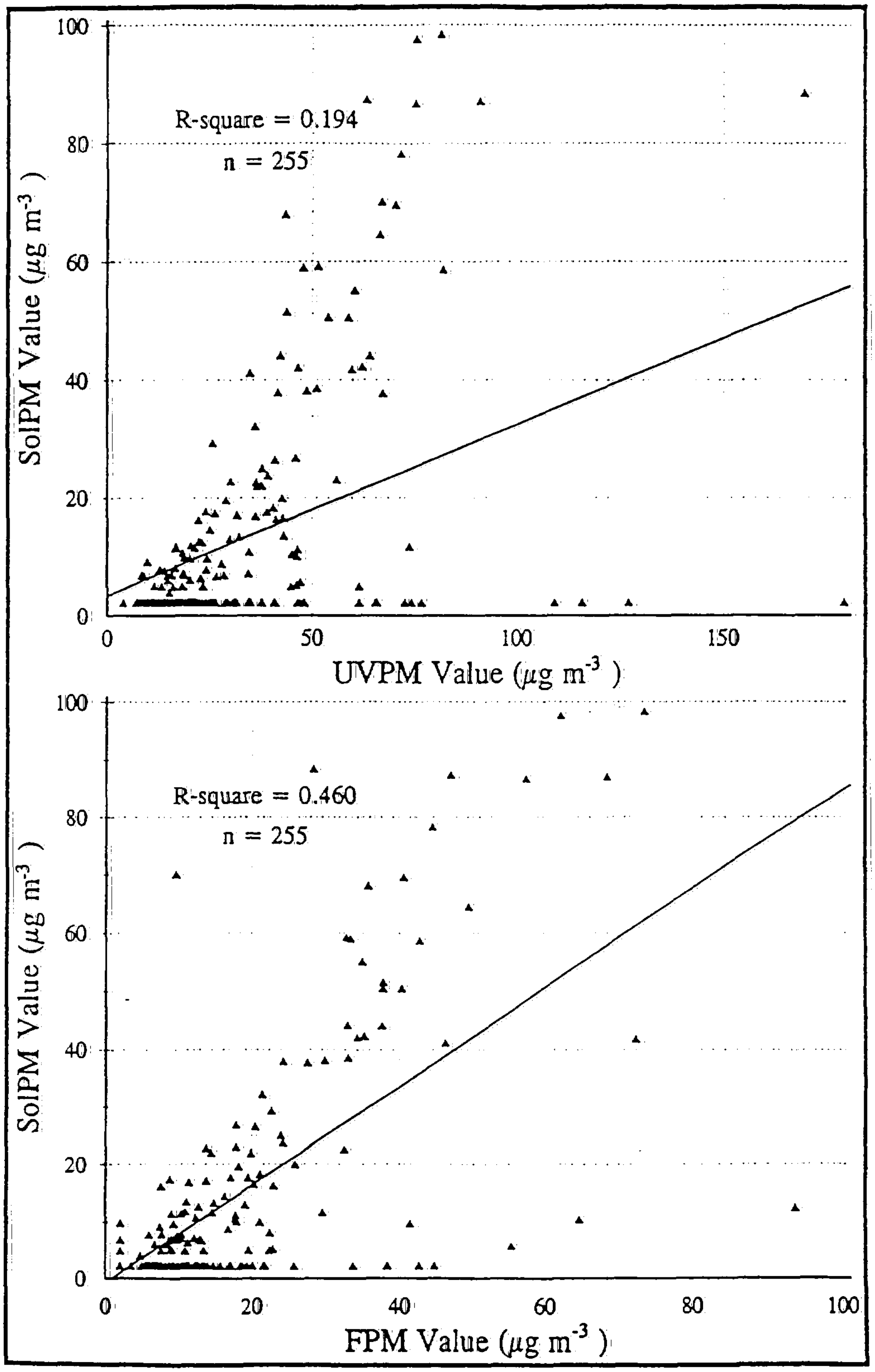

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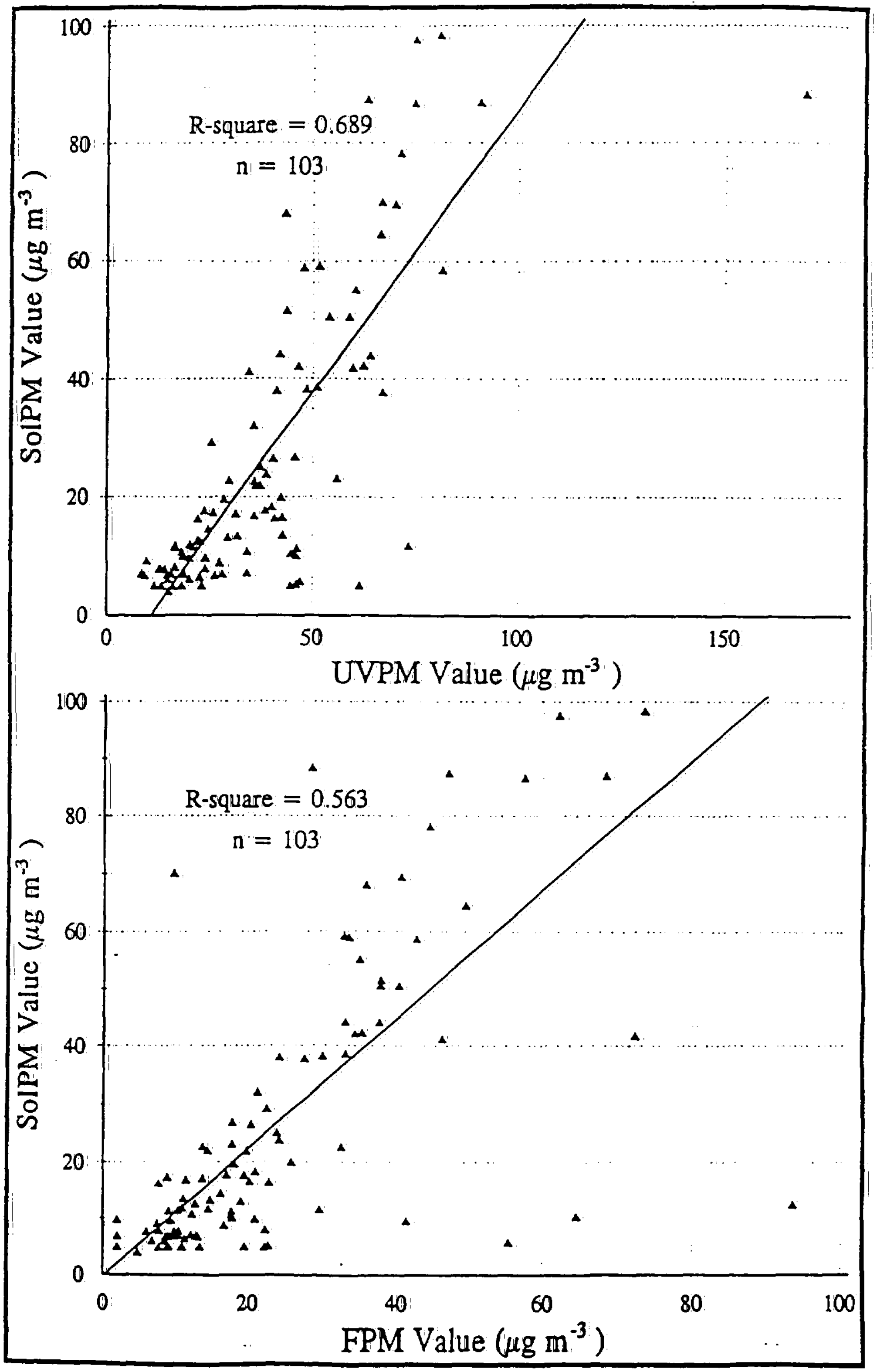
Figure 10

CORRELATION OF SolPM RESULTS WITH UVPM and FPM after removal of SolPM = 2.0 $\mu g\ m^{-3}$



SECTION 3

ETS AND LUNG CANCER

MONITORING IN REAL LIFE SITUATIONS

2026224153

Am. Ind. Hyg. Assoc. J. 49(9):423–426 (1988) Sept. 3019

Concentrations of Nicotine, RSP, CO and CO_2 in Nonsmoking Areas of Offices Ventilated by Air Recirculated from Smoking Designated Areas

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The exposure of nonsmokers to environmental tobacco smoke (ETS) when smoking is relegated to designated areas that are not separately ventilated is of considerable interest. Concentrations of nicotine, respirable suspended particles (RSP), carbon monoxide (CO), and carbon dioxide (CO_2) were measured in offices under different conditions of smoking regulation: smoking prohibited; smoking prohibited areas receiving recirculated air from designated smoking areas; smoking and nonsmoking sections of these designated smoking areas. Nicotine was collected by pumping air for periods of 1–8 hr at 1 L/min through sampling tubes containing a styrene divinylbenzene copolymer. RSPs (5 μm cut-off) were measured using an optical side scattering instrument. CO was measured by a direct reading electrochemical analyzer and CO_2 by colorimetric detector tubes. Detection of nicotine in nonsmoking office areas that received recirculated air from smoking designated areas required sampling times of 4 hr or more. Nicotine levels in such offices were approximately 1.0 $\mu g/m^3$. RSP, CO and CO_2 concentrations were approximately the same in these offices as compared to nonsmoking offices not exposed to recirculated air from smoking areas. Providing a designated but not separately ventilated smoking area appears to be effective in eliminating most components of ETS from nonsmoking office work areas.

Introduction

A number of municipalities (San Francisco and Vancouver being leading examples) have passed bylaws to regulate smoking in public buildings. In principle these bylaws apply to public buildings and places of employment and establish a norm of no smoking except in *smoking areas designated* by the employer or proprietor. The Canadian and American Federal Governments are preparing to develop approaches to regulate smoking in workplaces under federal jurisdiction. Provincial and state governments are making similar preparations.

Four options are available to regulate office smoking:

1. Prohibiting smoking outright;
2. restricting smoking to designated areas that are ventilated separately;
3. restricting smoking to designated areas that are not ventilated separately; and
4. *providing some framework* by which an adjustment between smoking and nonsmoking workers may be achieved without directly regulating the placement of smokers.

The third option, that of providing a designated but not separately ventilated smoking area, appears to be the most frequently adopted procedure. A certain proportion of a building's population will demand a location where they may be allowed to smoke (for example, employees on their coffee and lunch breaks, members of the public waiting for services, or persons who are residents of the building—such as in prisons or hospitals.) Governments and the private sector own, operate and rent a wide variety of different buildings, however, most of these buildings do not offer separate ventilation for different locations. To provide separate ventilation would not only be costly in many instances but very often physically impossible. Thus, the least *disruptive* and costly solution for many buildings appears to be the setting aside of designated but not separately ventilated smoking areas.

A question of considerable interest is the extent to which designated but not separately ventilated smoking areas are effective in decreasing exposure to environmental tobacco smoke (ETS) in nonsmoking areas. This project was designed to provide some information on that question.

The authors report here the outcome of a series of measurements of nicotine, respirable suspended particles (RSP), carbon monoxide (CO) and carbon dioxide (CO_2) obtained in the following locations:

1) two cafeterias, each having smoking and nonsmoking areas;
2) four nonsmoking floors which received air recirculated from a ventilation system common to one of the cafeterias; and
3) two nonsmoking offices with independent ventilation systems which, therefore, did not receive air recirculated from designated smoking areas.

Methods

Air sampling for nicotine, RSP, CO and CO_2 and an observation of the number of office occupants present and cigarettes smoked was undertaken in two adjacent buildings

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(Vancouver City Hall and City Hall Annex): Building A, which is a sealed, mechanically ventilated building, and Building B, which has opening windows and mechanical ventilation only in selected areas.

Building Description

Building A is a 4-story sealed office building with 2 levels of underground parking. Each of the 4 floors contains approximately 1390 m^2 (15 000 ft^2) of office space. Fresh air from an intake at ground level is supplied to an air-handling unit in the basement mechanical room. This fresh air is filtered, conditioned and then supplied unmixed to air induction units located at exterior walls. Air is returned, via ceiling return grates, to a second air-handling unit in the basement, which exhausts a portion of the return air, adds makeup air (minimum of 20%), and filters, conditions and returns the air to the occupied space via ceiling diffusers. As a result, indoor air from different parts of the building and different floors is mixed. Smoking is prohibited in all work areas and public areas of the building and is permitted only in the smoking section of the fourth floor cafeteria which is not separately ventilated.

Building B is a 12-story, unsealed building with opening windows and, originally, no mechanical ventilation system. Most areas are passively ventilated by building leakage while separate ventilation systems have been incorporated in only a few areas. In the offices where measurements were taken, rooms with exterior walls have opening windows. Additional ventilation is supplied to the central zone of each of these offices by an air-handling unit which receives fresh air from an intake at ground level. The zone air-handling unit feeds conditioned air to a supply-air plenum (in the ceiling space) where individual fan-coil units temper the air again and deliver it to the occupied space below. Air which has not been exhausted through windows or doors is returned to the ceiling plenum and again tempered by the fan-coil units. These systems, therefore, have no ducting common to other areas of the building. Smoking is prohibited in all work areas and public areas in the building except the smoking section of the cafeteria (which is located in the basement). Heated/cooled air is supplied separately to the cafeteria and exhausted through windows.

Sampling and Occupant Observation

Three samples were taken in each of the smoking and nonsmoking sections of the cafeterias of Buildings A and B; two samples on each of the four floors in nonsmoking offices of Building A; two samples in the nonsmoking offices of Building B; and two samples of RSP outdoors.

Samples for nicotine were obtained using a portable air sampling pump housed inside a briefcase. Because of the effect of air sampling on occupant behavior,[11] the sampling apparatus was designed to collect samples in an unobtrusive manner. Nicotine samples were collected by pumping air at 1 L/min through sorbent tubes containing XAD-4 resin, a styrene divinylbenzene copolymer. The sorbent tubes contained 80 mg of resin in the front (primary) section and 40 mg in the rear (secondary) section. Samples were collected for 1 hr each in the cafeteria locations and for periods of 2, 4 or 8 hr at other sampling sites. Respirable suspended particles (3 μm cutoff) were determined using a P-5H digital dust indicator (Sibata Scientific Technology, Tokyo, Japan) which measures light side-scattered by suspended particles. The unit was calibrated at the factory to monodispersed stearic acid particles with a mean diameter of 0.3 μm. The unit usually measured respirable particles for the entire sampling period, depending on battery charge. Approximately midway into the 1- or 2-hr air sampling period, CO and CO_2 concentrations were measured at the sampling locations. (CO and CO_2 were measured more often during 4- and 8-hr sampling periods.) CO was measured using a direct-reading electrochemical analyzer (Nova 310 L, Nova Analytical Systems, Inc., Hamilton, Ontario) housed in a flight case. CO_2 was measured using colorimetric detector tubes (Gastec, Gastec Corporation, Yokohama, Japan) and a manual sampling pump.

During a sampling period, the number of occupants in a predefined observation area and the number of cigarettes smoked in that area were observed and recorded. The observation areas were defined by visual configurations and reliability of surveillance of the observable office area. For purposes of comparison, number of persons and cigarettes smoked were calculated per 10 m^2 where applicable. At the completion of sampling, the sorbent tubes were refrigerated until analysis.

Analysis

In the chemical analysis of nicotine, resin beads in the sorbent tubes were transferred to gas chromatograph autosampler vials to which were added 50 μL of quinoline (100 mg/L) to serve as an internal standard and 1 mL of ethyl acetate as an extraction solvent. Triethylamine (0.01% by volume) was added to the extraction solvent to prevent adsorptive losses of nicotine onto the glass autosampler vials. Samples and spiked standards then were placed on an automatic shaking device and shaken for 30 min. A Hewlett-Packard Model 5880A or Model 5830A gas chromatograph equipped with a nitrogen-phosphorus detector was employed in conjunction with an autosampler and a GC terminal to determine peak areas of the nicotine and compare them with the areas obtained from nicotine standards. The assayed nicotine was corrected for the desorption efficiency (usually 94%) of the particular lot of XAD-4 resin used in sampling. Final nicotine results were divided by the volume of air sampled to yield results in μg/m^3. The rear (backup) sections of sorbent tubes were analyzed separately and, except for one case, always yielded nicotine determinations less than the limit of detection, thus indicating no breakthrough of nicotine past the primary section. [The authors' procedure, by and large, is based on the National Institute of Occupational Safety and Health (NIOSH) method.[12]]

Respirable suspended particles were estimated by converting the digital counts of particles per sampling time to an average count per minute. A background count of 5 counts per min was subtracted from the average to yield RSP values in μg/m^3.

Results

Table I summarizes measurements for RSP, CO, CO_2, nicotine; average number of persons per 10 m^2; and average number of cigarettes smoked per hour per 10 m^2 (where applicable). Because of the large variability and suspected skew of measures, means, medians and ranges are given. Measurements in the cafeteria smoking areas each are based on 6 samples as are measurements in the cafeteria nonsmoking areas. Because there were no perceptible differences between cafeterias in Buildings A and B, both for smoking and nonsmoking areas, their data have been merged. Measurements in nonsmoking office areas in Building A are based on 8 samples, and measurements in nonsmoking areas in Building B are based on 2 samples.

There were significantly more persons per unit area in the cafeterias than in the nonsmoking offices. The numbers of individuals per 10 m^2 in smoking and nonsmoking areas of cafeterias, however, were approximately the same. As might be expected, both CO and CO_2 levels were higher in the smoking than nonsmoking areas of the cafeterias. This also was true for RSPs. Nicotine levels averaged 14.0 $\mu g/m^3$ in the smoking area and 6.2 $\mu g/m^3$ in the nonsmoking area of the cafeterias. The drop in RSPs and nicotine from smoking to nonsmoking areas of the cafeterias is quite steep and attests to the rapid dilution of ETS.

Contributions to RSP, CO and CO_2 that are caused by smoking in the designated smoking area are diluted further in the recirculated air. This dilution can be seen from a comparison of measurements in the office areas of Building A with Building B. Concentrations of RSP, CO and CO_2 in Building A's nonsmoking areas, which received recirculated air from the smoking area, are approximately the same as those measurements taken in Building B, which did not receive any such recirculated air (also see Table II). Of special interest are measurements of nicotine. It is important to keep in mind that the detection of nicotine in air, in the dilute quantities in which it may be present, requires a lengthy sampling procedure. As the concentration of nicotine in air decreases, larger air samples must be obtained to detect that concentration. For the method used here, a 2-hr sample at 1 L/min would detect nicotine concentrations greater than 0.8 $\mu g/m^3$. Of 4 samples taken for 2 hr each, not a single sample detected a concentration above 0.8 $\mu g/m^3$. For a 4-hr sample at 1 L/min, the lower level of detection is 0.4 $\mu g/m^3$. At that level, 1 positive detection at a concentration of 1.0 $\mu g/m^3$ was made in 1 out of 3 samples. For the 1 sample taken for 8 hr, the lower level of detection was 0.2 $\mu g/m^3$. That sample measured a concentration of 0.8 $\mu g/m^3$ (findings summarized in Table II).

Discussion

Studies of office air quality have demonstrated that significant reductions in ETS related RSP may be achieved in nonsmoking areas when smoking is limited to designated areas that are not ventilated separately.[1] The extent of involuntary exposure to ETS, however, best may be established quantitatively when nicotine is used as the marker.* It has been suggested[2] that advances in measurement technology may provide grounds for reliance on nicotine as a general indicator of ETS. Other components of ETS may be less useful for developing an ETS exposure index. ETS components are complex and variable and also include many constituents similar to those emitted from other sources.[10]

*The observation that nicotine in sidestream smoke is mainly in the vapor phase while in mainstream smoke it is more in the particulate (deposit) phase poses no obstacle to the use of nicotine as an index of ETS infiltration because building occupants are not exposed to mainstream smoke unless they actively do smoke. The nicotine concentration obtained from sampling the air is a representative sample of ambient ETS inhaled by nonsmokers.

TABLE I
Comparison of ETS Related Air Quality Parameters in Nonsmoking Work Areas and Designated Smoking Areas

		RSP[A] ($\mu g/m^3$)	CO (ppm)	CO_2 (ppm)	Nicotine ($\mu g/m^3$)	Persons /10 m^2	Cigarettes /hr/10 m^2
Smoking areas of Cafeterias A & B combined	Mean	70	3.9	600	14	1.8	1.2
	Range	23–129	1.1–11.4	450–1000	<1.8–43.7	0.78–3.42	0.53–1.67
	Median	74	2.5	660	11	1.8	1.2
Nonsmoking areas of Cafeterias A & B combined	Mean	32	2.6	560	6.2	1.7	
	Range	15–57	1.2–4.5	400–700	<1.8–10.9	0.78–2.5	NA[B]
	Median	26	2.4	580	7.9	1.7	
Nonsmoking office area, Building A	Mean	6	1.8	490		0.73	
	Range	4–11	1.3–2.3	400–580	C	0.29–1.9	NA
	Median	6	1.7	500		0.48	
Nonsmoking office area, Building B	Mean	7	1.36	450		0.9	
	Range	6–8	1.3–1.4	400–500	C	0.53–1.28	NA
	Median	7	1.36	450		0.9	

[A]Mean outdoor RSPs were 10 $\mu g/m^3$.
[B]NA = not applicable
[C]See Table II

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TABLE II
Nicotine, RSP, CO and CO_2 Concentrations in Eight Locations in a No Smoking Office Area that Receives Recirculated Air from a Smoking Designated Area and in Two Locations without Such Recirculation[A]

	Location	Sample Time (hr)	Nicotine ($\mu g/m^3$)	RSP ($\mu g/m^3$)	CO (ppm)	CO_2 (ppm)	Persons /10 m^2
Recirculated air	1	2	<0.8	6	1.7	580	0.80
	2	2	<0.8	5	2.3	500	0.42
	3	2	<0.8	5	1.3	400	1.90
	4	2	<0.8	4	2.0	500	0.36
	5	4	<0.4	11	2.2	550	0.30
	6	4	<0.4	5	1.7	450	0.28
	7	4	1.0	6	1.7	500	1.02
	8	8	0.8	6	1.6	450	0.96
No recirculated air	9	B	B	8	1.4	400	0.53
	10	B	B	6	1.3	500	1.28

[A]Sampling time for nicotine ranged from 2 to 8 hr.
[B]Nicotine was not measured because these offices could not receive ETS from any source.

Air sampled for 2 hr at 1 L/min (using the NIOSH protocol) reliably measures nicotine levels that are larger than 0.8 $\mu g/m^3$. Levels of nicotine appear to be at or below that concentration in offices in which smoking is prohibited but which receive air recirculated from smoking designated areas. To give meaning to such trace values, the exposure of an office worker to nicotine at 1 $\mu g/m^3$ for 1 hr can be calculated roughly. Given a breathing rate of 0.48 m^3/hr for the level of activity required during normal office work,[4,5] an office worker would breath air containing 0.48 μg of nicotine in 1 hr. This quantity is approximately equivalent to 1/1800 of the nicotine inhaled by actively smoking 1 cigarette (900 μg/cigarette[6]). Until relatively recently, calculations of a smoker's exposure to cigarette smoke was limited to amounts of materials in the mainstream smoke. Insofar as smokers are spatially close to their cigarette and often inhale relatively undiluted sidestream smoke, existing estimates of smokers' exposure to any component of ETS must be lower than their actual magnitudes. Thus, the nonsmoker probably inhales less than 1/1800 of the nicotine inhaled by a smoker when actively smoking one cigarette, unless this nonsmoker should be standing in very close proximity to a burning cigarette.

Based on these findings, it is the authors' belief that the provision of a designated smoking area appears to be effective in eliminating most traces of ETS from the rest of the office space, even if the designated smoking area is not separately ventilated. An exclusive reliance on regulating smoking while ignoring all other problems besides smoking which may influence the quality of air in the nonindustrial work environment may accomplish little in addressing indoor air quality problems, however, especially in so-called "sick buildings."[7]

If a designated area is made available for smoking in offices where otherwise smoking is not permitted, the designated space should be sufficiently large to prevent overcrowding.

Acknowledgment

Thanks are due to Mr. C. Collett for his help in arranging for sampling sites and to Mr. L. Strandebo for allowing access to the City Hall sampling sites. The authors are beholden to Dr. C. Nystrom for help with some of the equipment used for this work. Part of the costs of this project came from a special grant from the Council for Tobacco Research, Inc.

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Indoor Air Quality: The Contribution of Environmental Tobacco Smoke

R. Perry, J. N. Lester, M. Hunter, P. W. W. Kirk, and S.-O. Baek

Summary

An extensive 30-week survey of environmental tobacco smoke has been undertaken in Great Britain. The survey consisted of over 2,900 sampling operations according to a scheme which covered a range of situations to which the public are exposed during their travel and leisure as well as at home and work. Sampling took account of population distribution as well as geographical and seasonal effects.

Three components of tobacco smoke – particulate matter as measured by Minirams, carbon monoxide and nicotine – have been determined in smoking and non-smoking situations, whilst the reported presence or absence of smoking within 2 h prior to sampling was used to distinguish between non-smoking and smoking environments. The survey was structured around 30 min sampling periods, using unobstrusive, portable sampling equipment capable of detecting each of the three components at less than 5% of their individual (occupational exposure limit (OEL) is the UK recognized workplace safety level).

Overall mean Miniram particulate matter was 0.56 mg m^{-3}, with a smoking location mean of 0.81 mg m^{-3} and a non-smoking mean of 0.31 mg m^{-3}. These results, as determined by the Miniram light scattering device are, however, known to be an overestimate when compared to methods based on the measurement of particulate mass, such as the Piezobalance. Subsequent studies in a variety of locations have shown the Miniram to over-estimate by at least a factor of 2.5 in the presence of tobacco smoke.

The overall mean carbon monoxide level was 2.4 ppm with a mean of 2.7 ppm in smoking locations and 2.1 ppm in non-smoking locations. With the limitations of the carbon monoxide monitors, this difference is not thought to be significant.

In calculating the mean results for nicotine, a value of 6.8 $\mu g\, m^{-3}$ (i.e. half the limit of detection) was assumed whenever the nicotine level was below the limnit of detection. This has almost certainly led to an over-estimation of nicotine, particularly in non-smoking situations, where nicotine was rarely detected. Applying this factor the mean overall nicotine concentration was 14 $\mu g\, m^{-3}$ with a mean of 21 $\mu g\, m^{-3}$ in smoking locations and 8 $\mu g\, m^{-3}$ in non-smoking locations. No nicotine concentrations exceed the OEL set at 500 $\mu\, m^{-3}$ and 95% of all readings were below 10% of the OEL.

Introduction

Considerable effort has been expended over the last 15 years attempting to control emissions of air pollutants into the atmosphere from sources such as power stations, factories and automobiles [1]. More recently, increasing public awareness has prompted

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concern regarding the quality of indoor air, particularly as the majority of the population spend up to 80%–90% of their lives indoors [2].

There are many sources of indoor pollutants (both gaseous and particulate) including the use of gas stoves and fires, coal, coke, and wood fires, house plants, cooking, cleaning, painting, and the adoption of a variety of household and office products including cleaning agents, glues, correction fluids, plastics and varnishes [3, 4]. In addition, the simple act of movement resuspends particulate matter [5] whilst building materials and furnishings, especially when new, may release a variety of organic materials into the indoor atmosphere [6]. Release of formaldehyde from cavity wall insulation, furniture and fabrics are all examples of such indoor air pollutants and are of considerable public concern.

Specific interest has been directed towards pollutants associated with emissions from gas cooking and other problems such as radon build up, "sick-building" syndrome and environmental tobacco smoke (ETS) [1, 7]. Probably the most emotive issue is that of ETS, largely with respect to considerations of irritation and discomfort, but more especially in the light of recent epidemiological studies alleging risks to the health of the exposed non-smoker.

The contribution ETS components make to the indoor air environment is difficult to quantify for a number of reasons. Environmental tobacco smoke has not yet been sufficiently characterized such that its nature can be clearly defined. The concentration of any individual ETS compound or group of compounds in an enclosed space is dependent upon its generation rate from the tobacco, the source consumption rate, ventilation, the concentration of the constituent in the incoming ventilation air, dimensions of the room, the degree of mixing, the rate of removal by adsorption or chemical transformation and the effectiveness of any air cleaning devices such as air conditioning systems [8].

One major problem when attempting to define the contribution of ETS to air quality is that in real life situations ETS normally exists in association with a complex mixture of air contaminants from other sources, particularly those from other combustion sources [1]. Indeed, these may not necessarily originate from indoor situations. Pollutants such as carbon monoxide for example, readily pass from the outdoor to the indoor environment without significant change in concentration [9]. However, indoor pollutants can give rise to high local concentrations, but are greatly diluted on passing to the outdoor environment. In contrast, reactive gases such as ozone and sulphur dioxide are rapidly removed in the indoor environment and levels are normally only a fraction of those commonly encountered outdoors [10], thereby indicating the complex relationship between indoor and outdoor air.

To date the major short-fall of studies examining ETS in the failure to adequately quantify the actual ETS dose received by the non-smoker. Environmental Tobacco Smoke is a complex and greatly diluted mixture of sidestream (commonly defined as the smoke which issues from the product between puffs), mouthspill (smoke released from the mouth before inhalation) and exhaled smoke, the proportions of which will vary depending on the smoking behaviour of the individual smokers. In realistic circumstances ambient concentrations depend on sidestream smoke and the exhaled mainstream smoke [11, 12]. In order to quantify ETS components therefore, any component determined should ideally be unique to tobacco smoke, present in sufficient quantity to be easily detectable, similar in emission rate for a variety of tobacco products and be present in a fairly consistent ratio to other smoke components of interest [9]. Furthermore, it is apparent that in order to evaluate the contribution of tobacco smoke to indoor air quality, non-smoking as well as smoking situations need to be studied under as wide a variety of actual conditions as possible.

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Consequently the objectives of this study were

- to assess indoor air quality in home, work, leisure and travel situations;
- to evaluate the ETS components of indoor air;
- to compare smoking and non-smoking environments.

Materials and Methods

Markers for ETS

Three main components of tobacco smoke were identified as markers within the study, namely, nicotine, carbon monoxide and Miniram particulate matter (TPM).

Nicotine fulfils most of the criteria for a suitable marker being a major component of, and almost exclusive to, tobacco smoke and also is detectable in small quantities of air at low concentrations [11, 13, 14]. Moreover, nicotine has been measured in the majority of existing studies [1].

Carbon monoxide is a commonly measured constituent of indoor tobacco smoke in field surveys since it is a major component of cigarette smoke and is relatively easy to measure, although there are many other sources of indoor carbon monoxide besides tobacco smoke [9, 11, 16].

Total suspended particulate matter can be defined as particles (generally $< 15\ \mu m$) suspended in the atmosphere, as collected for subsequent gravimetric determination. An alternative generalised measurement of particulates utilizing a light scattering technique was used and subsequently calibrated against piezobalance and gravimetric methods.

Survey Design

A 30-week field survey was designed to study four types of indoor environment; workplaces (W), homes (H), leisure (L) and travel (T). Monitoring was evently distributed between each type and performed by an independent research laboratory (Hazleton Laboratories UK Ltd., UK). The study was designed to evaluate a total of 30 locations representing a wide variety of exposures throughout the United Kingdom which were considered in three major regions according to population density (Table 1).

These figures were derived from recent statistics [17], by a market research group (MAS Survey Research Ltd., UK) to represent the geographical regions, urban and social status of the UK.

Homes were randomly selected after reclassification by local authority area according to these criteria. Work situations were randomly selected within each classification according to type and size (number of employees) of business to reflect any particular location based on a quota system. Leisure and travel situations were identified and arranged around work and home samples to maintain flexibility.

A balance of timing with respect to days of the week, start times, and times of the year was arranged so as to cover the spectrum of normal life exposure (Fig. 1). The study was divided into three 10-week-periods, each area being sampled completely over any 10-week-period. Each operative subsequently rotated to a different area during the following 10-week-period. A staggered pattern of start times and days enabled extended coverage of time of day and days of the week. These were repeated by succeeding operatives.

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Table 1. Allocation of sampling locations in the United Kingdom

Area No.	Location	Population %	Total regions	Conurbations	Large urban	Small urban/rural	
						H[a]	L[b]
1	South East	30	9	4	1	3	1
2	South West, Wales, Midlands, East Anglia	34	10	1	2	3	4
3	North, Yorkshire, Humberside, North West, Scotland	36	11	6	2	1	2

[a] H – high density
[b] L – low density

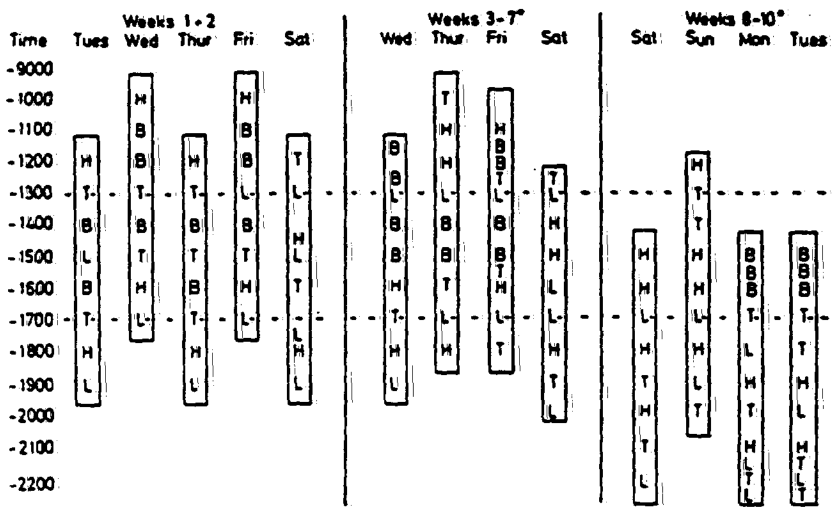

Fig. 1. Typical operative work schedule during any 10-week-period. *H:* house call; *B:* business call; *T:* travel call; *L:* leisure call; * Residual T & L performed where possible after arrival at new locality

Equipment Selection and Analytical Procedures

The equipment used was potable, robust and discretely operable, being reliable and precise. Four integrated kits were assembled and tested in field trials prior to the study. Each kit consisted of a carbon monoxide dosimeter (General Electric 15 ECCICO2; MDA Scientific, UK), a Miniram PDM-3 particulate dosimeter (GCA Corp., USA), a

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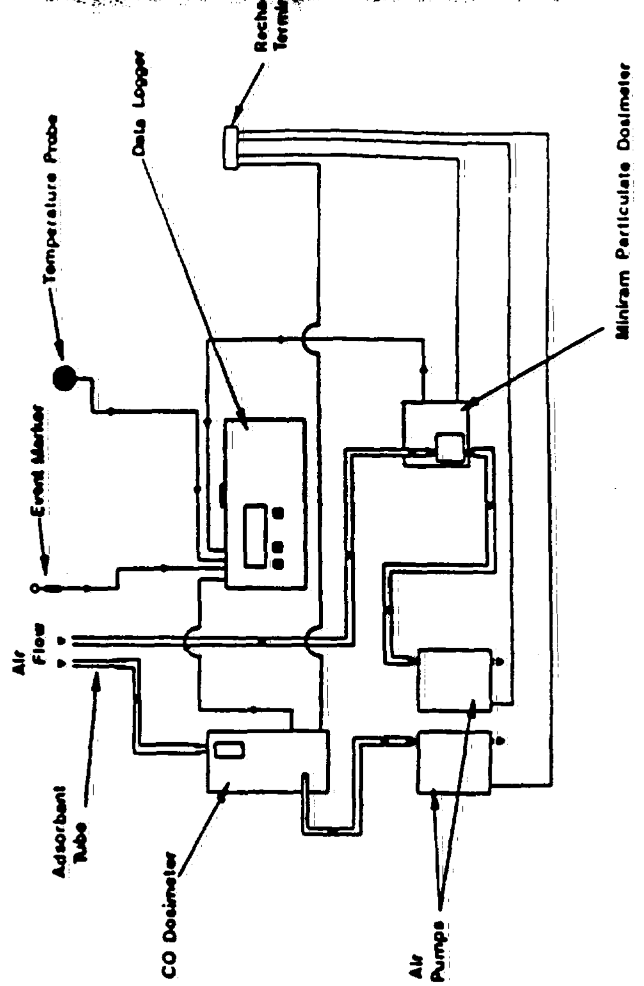

Fig. 2. Schematic of the portable sampling equipment

temperature probe and an electrical event marker interfaced to a data logger. Air flow through CO and TPM dosimeters during sampling was maintained at rates of 0.006 m^3 h^{-1} and 0.015 $m^3 h^{-1}$ respectively using two Alpha pumps (DuPont Ltd., USA) illustrated in Fig. 2.

Each sampling event lasted for a period of 30 min, CO, TPM and temperature readings being logged every 2 min. Concurrently, nicotine samples were obtained by adsorption onto 200 mg of Tennax TA adsorbent (Chromopak, UK) contained in open ended steel tubes which were placed on the CO line. Readings of relative humidity were recorded manually along with relevant site details in the operators diary. Nicotine

samples were analysed using 2-stage thermal desorption followed by capillary gas chromatography (Perkin Elmer, 8320) fitted with FID.

A total of 5% of samples were validated in the field by Imperial College staff in parallel with Hazleton field operatives. Instrument cross checks were also performed with all kits between each 10-week-work-period.

Miniram TPM dosimeters were retrospectively subject to a separate calibration exercise in comparison with piezobalance and gravimetric methods.

Results

Summary data for the complete 30-week-study is given in Table 2 along with corresponding percentile values. In each table a smoking sample is one in which smoking is known to have taken place during sampling or within the 2 h prior to sampling.

Carbon monoxide had an overall mean concentration of 2.4 ppm for some 2,657 measurements throughout the survey. In smoking situations the mean carbon monoxide level was 2.7 ppm (49.6% of samples) and in non-smoking situations it was 2.1 ppm. In 95% of cases where smoking was taking place, carbon monoxide concentrations were less than 7.2 ppm compared with 5.9 ppm in non-smoking situations (Table 2). The distribution of CO values indicated the relatively high proportion of readings within the ranges 0–2 and 2–4 ppm (Fig. 3). Similarly mean CO values observed in each activity confirmed these low levels, travel locations having greatest means at 2.9 ppm and 2.7 ppm for smoking and non-smoking situations respectively (Fig. 3). However, differences between these situations remained small regardless of activity or smoking status.

When comparing the difference in particulate levels between smoking and non-smoking situations the values quoted should be assessed with caution due to the likely over-estimation of the Miniram for particulate levels in mixtures of ETS and particulates from other sources. Indeed, comparative assessments of Miniram TPM, piezobalance and gravimetric methods revealed overall overestimates of 2.5 over piezobalance data and 2.0 over gravimetric data. These also varied according to smoking status. The mean overall Miniram TPM reading determined from 2801 readings was 0.56 mg m^{-3}. In places where smoking occurred a mean of 0.81 mg m^{-3} was found whereas a mean of 0.31 mg m^{-3} occurred where no smoking was recorded. Smoking and non-smoking related uncorrected Miniram TPM values in 95% of all cases were less than 2.42 and 0.88 mg m^{-3} respectively. A similar distribution of data was observed with low numbers of high readings although this was less marked in smoking situations (Fig. 3). The highest mean TPM values were observed in smoking locations particularly in leisure areas with a value of 0.91 mg m^{-3} and in travel locations with a mean of 0.79 mg m^{-3} (Fig. 3).

Of the 2912 sites sampled for nicotine, 49.6% represented smoking situations. Across these samples the mean nicotine concentration was 14 μg m^{-3} (median N.D.) compared with 21 μg m^{-3} in smoking locations only and non-detectable in non-smoking situations (Table 2), their distribution is shown in Fig. 3.

Nicotine was not detected in 77.5% of all samples taken. Concentrations found were less than 49.8 μg m^{-3} in 95% of all samples whereas in smoking situations 95% were below 74 μg m^{-3}. Where no smoking occurred within 2 h of sampling, 95% of samples were below 16 μg m^{-3}, and nicotine exposure was consistently very low across all activities. Where smoking occurred travel and leisure activities appeared to be associated with highest nicotine readings, mean nicotine values being 24 and 22 μg m^{-3} respectively (Fig. 3).

Nicotine, carbon monoxide and miniram TPM samples recorded during the 5% validation programme, initially randomly chosen and subsequently selected, were

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Table 2. Summary and percentiles for 30-week-study

Activity		Temp. °C	R.H [%]	CO (PPM) SM(T)	CO (PPM) SM(Y)	CO (PPM) SM(N)	TPM (MG/M3)* SM(T)	TPM (MG/M3)* SM(Y)	TPM (MG/M3)* SM(N)	Nicotine (UG/M3)** SM(T)	Nicotine (UG/M3)** SM(Y)	Nicotine (UG/M3)** SM(N)
Travel	mean	20	44	2.8	2.9	2.7	0.62	0.79	0.42	17	24	7
	sd	5	8	2.8	2.5	3.1	0.64	0.75	0.36	31	40	4
	min	1	22	0.0	0.0	0.0	0.00	0.00	0.07	7	7	7
	max	34	75	17.4	13.1	17.4	4.98	4.98	1.83	414	414	42
	data	545	308	518	283	235	538	297	241	564	313	251
Work	mean	20	45	2.1	2.2	2.1	0.41	0.61	0.31	10	14	9
	sd	4	9	2.7	3.3	2.4	0.42	0.59	0.26	12	18	7
	min	8	23	0.0	0.0	0.0	0.00	0.07	0.00	7	7	7
	max	30	75	31.9	31.9	21.9	5.78	5.78	2.20	167	167	99
	data	723	721	671	221	450	704	224	480	733	238	495
Home	mean	20	46	1.9	2.3	1.8	0.36	0.70	0.27	10	19	8
	sd	3	9	2.3	2.9	2.1	0.36	0.52	0.23	17	33	6
	min	10	5	0.0	0.0	0.0	0.00	0.07	0.00	7	7	7
	max	30	71	26.2	26.2	25.4	3.15	3.15	2.05	292	292	82
	data	766	763	688	139	549	748	156	592	774	162	612
Leisure	mean	20	45	2.7	2.8	2.2	0.84	0.91	0.33	20	22	8
	sd	3	8	2.7	2.7	2.6	0.82	0.85	0.26	29	31	6
	min	8	17	0.0	0.0	0.0	0.07	0.07	0.07	7	7	7
	max	32	75	28.7	28.7	18.9	6.22	6.22	1.24	450	450	66
	data	819	578	780	676	104	811	703	108	841	729	112
Total	mean	20	45	2.4	2.7	2.1	0.56	0.81	0.31	14	21	8
	sd	4	9	2.7	2.8	2.5	0.63	0.77	0.27	24	32	6
	min	1	5	0.0	0.0	0.0	0.00	0.00	0.00	7	7	7
	max	34	75	31.9	31.9	25.4	6.22	6.22	2.20	450	450	99
	data	2,853	2,370	2,657	1,319	1,338	2,801	1,380	1,421	2,912	1,442	1,470

Note *: Particulate matter measured by miniram
**: Nicotine data below detection limit included as 6.8 UG/M3

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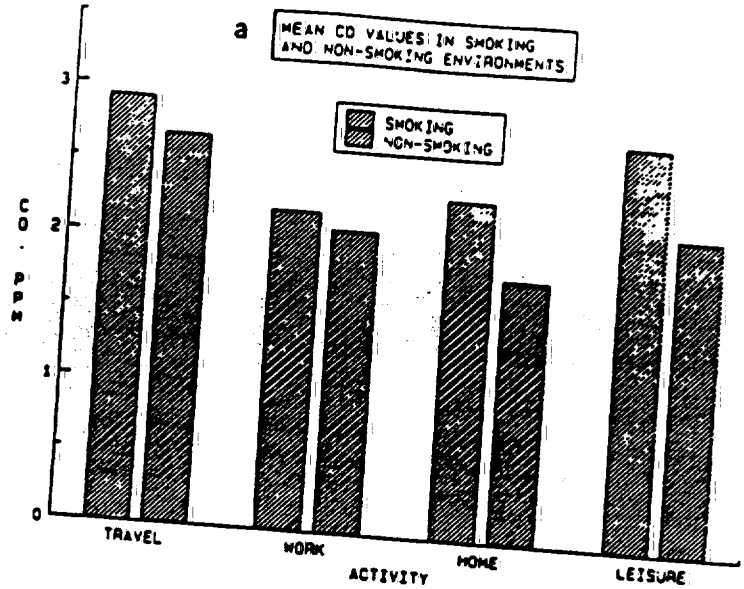
Table 2 (continued)

Percentiles	CO(PPM)			TPM(MG/M3)			Nicotine(UG/M3)*		
	SM(T)	SM(Y)	SM(N)	SM(T)	SM(Y)	SM(N)	SM(T)	SM(Y)	SM(N)
Minimum	0.0	0.0	0.0	0.00	0.00	0.00	N.D	N.D	N.D
01% value	0.0	0.0	0.0	0.07	0.07	0.07	N.D	N.D	N.D
05% value	0.0	0.0	0.0	0.07	0.15	0.07	N.D	N.D	N.D
10% value	0.0	0.0	0.0	0.15	0.22	0.15	N.D	N.D	N.D
25% value	0.5	0.8	0.3	0.22	0.37	0.15	N.D	N.D	N.D
50% value	1.9	2.1	1.6	0.37	0.59	0.22	N.D	N.D	N.D
75% value	3.4	3.8	3.1	0.66	1.02	0.37	N.D	23.3	N.D
80% value	3.9	4.2	3.5	0.81	1.17	0.39	15.2	28.4	N.D
90% value	5.1	5.6	4.5	1.24	1.68	0.59	30.7	48.5	N.D
95% value	6.8	7.2	5.9	1.68	2.42	0.88	49.8	74.4	16.4
99% value	11.5	12.6	11.5	3.07	3.81	1.46	112.4	146.0	36.1
Maximum	31.9	31.9	25.4	6.22	6.22	2.20	449.9	449.9	98.5
No of data	2,657	1,319	1,338	2,801	1,380	1,421	2,912	1,442	1,470

* Note: 77.5% of data is below detection limit



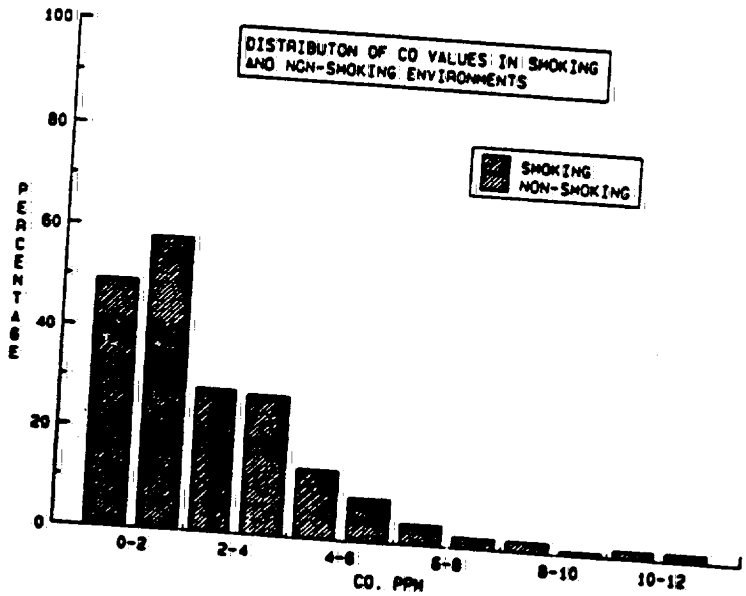


Fig. 3a–c. Mean values and distributions of CO (a), nicotine (b), and TPM (c)

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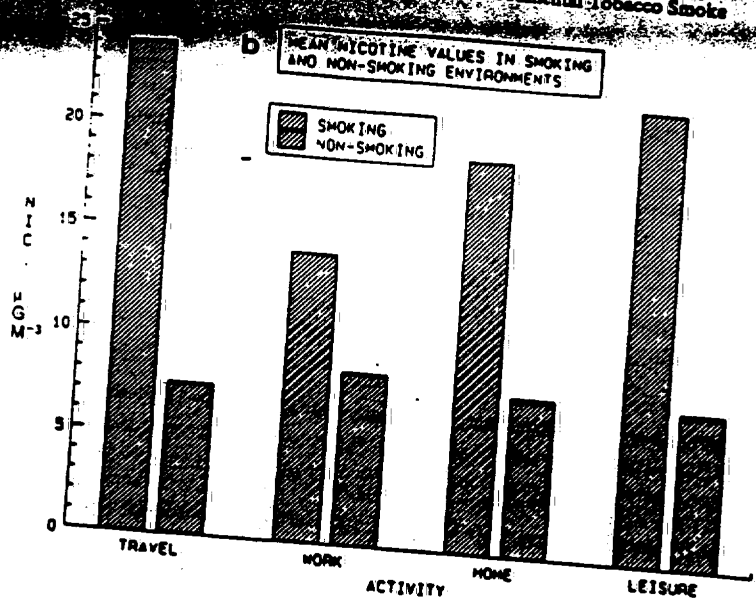

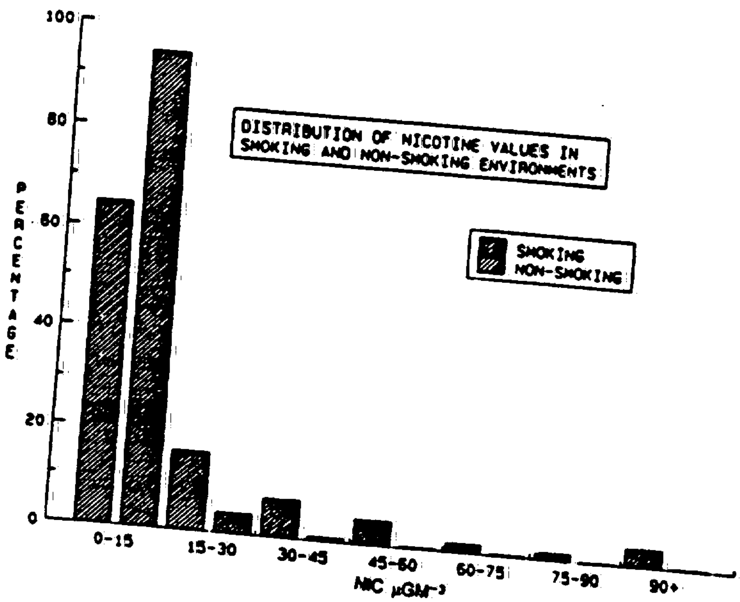

Fig.3b

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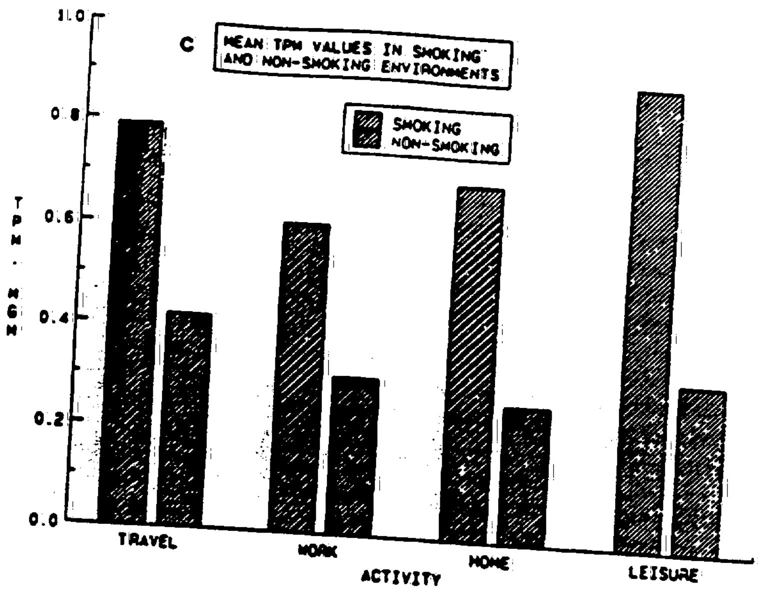

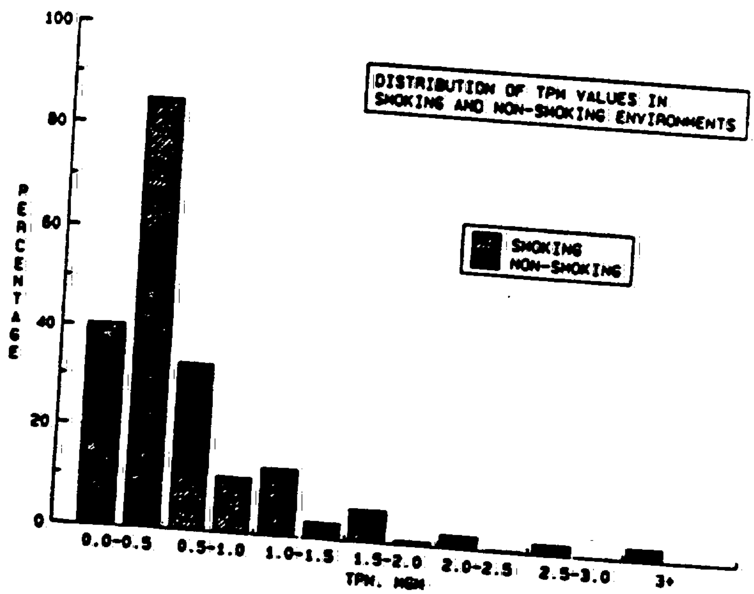

Fig. 3c

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subjected to paired t-test analysis. No significant differences were observed ($p = 0.05$) between Imperial College and HUK collected data for any parameter. Of the manually collected relative humidity and temperature readings which were t-tested, only temperature readings were found to be significantly different ($p = 0.05$). However, the difference (0.7°C) was negligible, subsequent tests performed in the laboratory confirmed the kits to be within 0.1°C of each other. Cross checks involving all kits between 10-week-periods also revealed no significant differences ($p = 0.05$) between paired nicotine, carbon monoxide and miniram TPM data.

Discussion

Several authors have previously indicated the inherent difficulty associated with the assessment of human exposure to ETS as a consequence of the number of variables involved [11, 15]. Apart from human behaviour and environmental factors, experimental design and analytical considerations are also important. In this study reported values are based on arithmetic means of large numbers of individual results and hence may reflect any imbalance that exists in the number of observations in each sampling category. The incidence of smoking and non-smoking samples within each category and activity may not reflect the "natural" relative incidence. Therefore an average exposure to ETS in all situations over both smoking and non-smoking samples require certain assumptions to be made prior to evaluation.

In terms of the survey as a whole the sampling locations within each activity were selected at random and therefore the relative incidence of smoking to non-smoking situations should reflect the "natural" incidence due to the large number of data points. Overall 49.5% of all locations were smoking samples. As one examines the sampling locations within each activity in more detail, however, the incidence of smoking to non-smoking may not reflect the "natural" incidence. For example, assessing the exposure to ETS in a bus in all situations is difficult since smoking occurred during or prior to 75% of the samples ($n = 113$). Over all travel locations, however, smoking had occurred in 56% of all samples ($n = 537$) a proportion which is more likely to be representative. Care must therefore be exercised when examining the data in detail.

No ambient air quality standard exists for nicotine in air in the United Kingdom but an OEL of 500 $\mu g\ m^{-3}$ has been set by the Health and Safety Executive for long term industrial exposure in terms of an 8 hour time weighted average. Of all the locations sampled in this study, 95% were less than 10% of this OEL, furthermore, the overall respective non-smoking and smoking nicotine means of 8 and 21 $\mu g\ m^{-3}$, were regarded as higher than anticipated in reality. These high mean values were attributable to non-detected nicotine readings, in non-smoking and smoking environments, being recorded as 6.8 $\mu g\ m^{-3}$ accounting for a reasonable distribution of data below the 13.6 $\mu g\ m^{-3}$ detection limit.

The mean overall nicotine value of 14 $\mu g\ m^{-3}$ was lower than that observed by Maramatsu et al. [18] who found a mean nicotine concentration of 20.3 $\mu g\ m^{-3}$ (max 83 $\mu g\ m^{-3}$, $n = 91$) in various work, leisure and travel locations. However, Sterling et al. [1] in a summary of 230 studies undertaken in buildings in the United States reported a median nicotine concentration of 8.5 $\mu g\ m^{-3}$ where smoking was permitted. Similarly, nicotine values associated with different activities are compatible with previous findings. For example, mean nicotine concentrations in offices of 19 $\mu g\ m^{-3}$, with maximum of 48 $\mu g\ m^{-3}$ from 10 samples and in railway workshops of 5.1 $\mu g\ m^{-3}$, maximum 41 $\mu g\ m^{-3}$ from 14 samples, have been measured [14]. An early study had

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suggested a mean nicotine concentration of 1.1 $\mu g\ m^{-3}$ for 160 samples with a maximum of 16 $\mu g\ m^{-3}$ [19].

Occupational Exposure Limits to carbon monoxide in the United Kingdom are 50 ppm as an 8 h time weighted average for long term exposure and 400 ppm as a 10 min time weighted average for short term exposure [16]. In this study carbon monoxide values were relatively low throughout and no individual 30 min mean carbon monoxide concentration exceeded the OEL of 50 ppm, and 95% of all locations were below 14% of this OEL. In 95% of cases where smoking was taking place, carbon monoxide concentrations were less than 7.2 ppm compared to 5.9 ppm in no smoking situations and the distribution data would indicate significant contributions from other combustion sources at the higher levels of carbon monoxide. Sisovic and Fugas [9] suggested that, during summer months, indoor carbon dioxide levels in shops can be significantly affected by proximity and density of traffic.

Particulates were determined by light scattering methods which must be related to standard gravimetric or piezobalance measurements due to difficulties in Miniram calibration related to particle size and particulate colour. Despite such calibration considerations the Miniram was the only instrument capable of the field monitoring required in this survey due to its portability, robust nature and logging capability. Comparative assessment of particulates were consistent with the findings of Rawbone et al. [20], who suggested a reduction factor of 2.5 for Miniram readings. Assuming a correction factor of 2.5, all particulate concentrations in the survey were below the OEL of 5,000 $\mu g\ m^{-3}$ set for "respirable dust" [21]. Furthermore, 95% of all samples would be less than 14% of the OEL and 95% of all smoking samples would be less than 19% of the OEL. The findings observed compare favourably with other studies when the correction factor is applied. Typical values for particulates in indoor air have been reported to range between non-detectable levels and 700 $\mu g\ m^{-3}$ in the United States with a median value of 37 $\mu g\ m^{-3}$ [1]. In a comparison of sampling methods for respirable suspended particulates (<3.5 μm) undertaken by the Reynolds Tobacco Company, Conner et al. [22] reported gravimetric particulate data up to 306 $\mu g\ m^{-3}$ in a restaurant where smoking was taking place.

Summary and Conclusions

- An extensive 30-week-survey of indoor air quality in the UK has been undertaken.
- Travel, work, home and leisure activities were evaluated approximately 3,000 times.
- Components of ETS monitored were: CO, Miniram particulate matter and nicotine in smoking and non-smoking situations.
- Ventilation and building design can affect ETS.
- TPM was significantly higher in smoking versus non-smoking situations although both are consistently less when determined by piezobalance and gravimetric methods.
- CO readings were all background level and significantly less than outdoor air. Fifty percent were below 2 ppm in smoking and non-smoking situations.
- More than 50% nicotine values in smoking and 90% in non-smoking environments were below the 14 $\mu g\ m^{-3}$ detection limit, 77.5% overall.
- Travel and leisure ETS exposures were consistently higher than home and work exposures.
- Home and work exposures to nicotine, 5% or less than UK long term occupational exposure limits (OEL), whereas travel and leisure were within 10% of UK OEL of 500 $\mu g\ m^{-3}$.

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THE MEASUREMENT OF ENVIRONMENTAL TOBACCO SMOKE IN 585 OFFICE ENVIRONMENTS

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In order to provide information on levels of environmental tobacco smoke (ETS) in office environments during 1989, a total of 585 offices was sampled for a number of factors, including respirable suspended particles (RSP), nicotine, carbon monoxide, carbon dioxide, room size, average number of room occupants, and number of cigarettes consumed. Each data set was collected over a one-hour sampling period. Discriminant analysis of the data collected showed a group of rooms used for light smoking (59.9% of total smoking rooms) was not significantly different from the nonsmoking rooms, in terms of the variables which contributed to the predictive ability of the model (RSP and nicotine). These light-smoking rooms overlapped somewhat with the heavy-smoking rooms, suggesting other variables not measured here might contribute to this model, such as air change rates or outside air intake volumes. This leads to the possibility that a range of smoker densities could be established inside which indoor air quality will not be significantly affected, thus reflecting the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) Standard 62-89, which shows that with good ventilation acceptable air quality can be maintained with moderate amounts of smoking. Statistical analysis also showed overall levels of ETS in offices to be considerably lower than estimated in work ten years previously, and that carbon monoxide is only weakly influenced by smoking activity. Carbon dioxide measurements taken in each room did not correlate significantly with RSP, nicotine, or carbon monoxide, and there were significant relationships between smoker density, RSP, and nicotine, respectively.

INTRODUCTION

Given the present-day concerns in the U.S. society about exposure to environmental tobacco smoke (ETS), it is important that measurements used to assess exposure to this substance are representative of conditions existing in modern office environments.

Numerous studies have measured levels of various components of ETS in both the home, workplace, and other public places. Repace and Lowrey (1980, 1982)

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presented results of field measurements made in non-office environments (bars, restaurants, bingo games, dinner dances, bowling alleys, sports arenas, waiting rooms, etc.), as well as experimental results in office type environments with high smoking rates (32 cigarettes smoked in 49 min). Respirable suspended particles (RSP) levels were found as high as 697 $\mu g/m^3$ in the non-office environments. In some office experimentation, an equilibrium of 1947 $\mu g/m^3$ of RSP was attained with a time constant (τ) of 14 min. They used data from these two papers to derive a mathematical model to estimate average RSP concentrations of 200 $\mu g/m^3$ in office environments where smoking was allowed (Repace and Lowrey 1987).

These papers are frequently referenced by other workers, and were extended by Repace and Lowrey to compute a "quantitative estimate of nonsmoker lung cancer risk from passive smoking" (1985a), and then "an indoor air quality standard for ambient tobacco smoke based on carcinogenic risk" (1985b). References to one or both of the first two papers are found within much literature on the subject of ETS that has been published since (Collishaw et al. 1984; Samet 1988; Meisner et al. 1989; Wells 1986; Sterling 1982; Wells 1989). These include some papers published as recently as 1989. In addition, the U.S. Environmental Protection Agency (USEPA 1990) currently includes references to these two papers in their Draft Guide to Workplace Smoking Policies. This document has not been formally released however, and is still in the review process.

There are no data in these papers on field measurements of ETS in office workplace environments; however, they have been referenced in many cases to argue that ETS is the major particulate component of indoor air, and hence smoking should be eliminated from the office environment. At present, research that measures components of ETS in discretionary smoking office environments has been limited—most studies contain small data sets thereby preventing precise statistical analyses. Examples of these relatively small scale ETS studies focusing on RSP where smoking was discretionary include Meisner et al. (1989), where RSP ranged up to 80 $\mu g/m^3$, with a mean of 34 $\mu g/m^3$. Also, Sterling et al. (1987) reported mean RSP levels of 37 $\mu g/m^3$ in smoking permitted areas in their Building Performance Database (BPD). Finally in this vein, Oldaker (1990) measured RSP levels in a range of offices and reported mean RSP levels of 126 $\mu g/m^3$ (mean Ultra Violet Particulate Matter [UVPM] levels of 27 $\mu g/m^3$). In any case, overall ETS levels have likely changed during the past decade due to improved ventilation rates and changing patterns of smoking in the U.S.

There is still a need for more study on all aspects of ETS in modern offices where smoking is allowed, especially in a larger variety of office environments sampled with the same methodology to highlight the influencing factors.

In an effort to provide contributory data on ETS components in office atmospheres, and to build on our understanding of which factors influence ETS levels, this study sets out to measure a series of parameters related to, or influencing, ETS in a very large sample of offices, using identical methodology in each. This provides us with up-to-date information on ETS in contrast with the data collected a decade previously.

METHODS AND MATERIALS

Obstacles which have prevented the collection of data as extensive as this before may have been: a) cost—since travel to and time in each office building constitutes a significant portion of a budget for this work; and b) access to each building. (Unless building owners or employers see some personal benefit, they are unlikely to allow their staff or tenants to be disturbed for air sampling exercises.) Each building requires individual persuasive visits allowing technicians into the building. This would normally be a prohibitive effort if the objective is to sample from hundreds of buildings.

Both cost and building access problems are eased by the nature of the indoor air diagnostic work routinely conducted by HBI. Access to many buildings is negotiated during the course of indoor air surveys allowing the ETS study to be added on to each routine indoor survey, with additional expense limited to a brief extra time period and analytical costs. In this manner, it was possible to survey several hundred buildings within practical cost constraints and without access difficulty.

During the visit to each building, the primary indoor air survey includes, as a minimum, visual inspections of the internals of each air handling system and measurements for a range of air contaminants throughout the building space. This survey is separate from the ETS study each building was subjected to, and no efforts are made in this paper to coordinate the results from the main air quality survey and the ETS studies.

The nature of this process dictates the buildings which were surveyed. Some were surveyed because of indoor air quality complaints by occupants. The majority were sampled, however, during the course

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of routine proactive monitoring visits to buildings perceived as generally healthy. We have no reason to suspect the buildings in this sample are not representative of office buildings throughout North America.

Unoccupied areas of buildings (e.g., areas under renovation), garages, stairwells, industrial areas, and outside terraces were avoided, although cafeterias and some areas designated as smoking lounges were included. A minimum of two ETS sample sets were taken in each building, and while it cannot be claimed that the occupants were unaware of the sampling activities, they were not informed that the sampling was related to ETS.

Data set collection procedures

A one-hour time period was allocated to each ETS sampling set. During this period, the field technician did not leave the space selected for sampling. Each sampling set consisted of the following information:

General:

Inspector I.D.
Client name
Building address
Overall building size
Number of stories

Specific to sampling set:

Type of business and work activity
Location (floor/room)
Room configuration details (partitions, supply, and return outlets)
Room size (m^2)
Number of people in room (average)
Number of cigarettes smoked
Respirable suspended particles ($\mu g/m^3$)
Carbon dioxide ($\mu L/L$)
Carbon monoxide ($\mu L/L$)
Nicotine ($\mu g/m^3$)
Temperature (°C)
Relative humidity (%).

Smoking density was then calculated for the hour sampling period by dividing the number of cigarettes consumed in the hour by the room size, to give $cig/m^2 \cdot h$.

The methodologies used for the air sampling were as follows:

Respirable airborne-particle counts were made using a piezoelectric microbalance that measured particles in the 0.01 to 3.5 µm size range. Flow rate through the piezobalance was periodically checked at 1 L/min with a bubble flow meter, and the sensor was cleaned with alcohol swabs after every five measurements. The unit is factory-calibrated with diluted welding fumes which have shown equivalence to indoor RSPs to ±10%. The lower detection limit was set at 10 $\mu g/m^3$.

Carbon dioxide levels were measured using a non-dispersive infrared absorption portable gas analyzer. Accuracy is ±2% over full scale. Periodic calibration of the instrument was with a factory-supplied span gas of 5000 $\mu L/L$ CO_2. Zero was set with dry nitrogen gas and the lower detection limit was set at 50 $\mu L/L$.

Carbon monoxide concentrations were measured using a controlled potential electrolysis detector, accurate to 10% full scale. Periodic calibration of the instrument was with a factory supplied span gas of 50 $\mu L/L$ carbon monoxide. The minimum detection limit was set at 1 $\mu L/L$.

Each of the above three parameters, as well as temperature and relative humidity, was measured in real-time and recorded ten times during the hour period. The real-time measurements and the average of the ten measurements were recorded in a standard field log, along with calibration data.

Airborne nicotine was measured after USEPA (1989) with a personal universal flow sampling pump drawing air through unfiltered XAD4 absorbent resin tubes. Samples were analyzed with gas chromatography. Results are expressed in total micrograms converted to $\mu g/m^3$, and the detection limit for our sampling rate of 1 L/min for a one-hour period is given as a conservative 1.6 $\mu g/m^3$ of air.

Statistical methods

General statistics. Statistical methods were used for the purposes of data description and correlation assessment between specific variables. Graphical methods were also used to evaluate relationships between specific variables.

The main goal of the statistical analysis was to evaluate differences between smoking-observed and nonsmoking-observed areas. To evaluate these potential group differences for variables such as RSP, nicotine, CO_2, and CO, t-tests were used.

Discriminant analysis. The goal of discriminant analysis (Karson 1982) was to predict group membership from several predictor variables. With these data, the discrete variable defining group membership was the type of room—either smoking-observed or nonsmoking-observed. Room type was entered into

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discriminant analysis with several predictor variables such as RSP and nicotine.

The discriminant analysis methodology used here is a stepwise procedure; it initially enters the most significant variable for predicting group membership, as defined by specific statistics, and proceeds to enter new variables into the model until the inclusion of additional variables does not increase prediction ability. There are other methods available that will determine the best predictor model, such as backward elimination. In many cases, however, each method will result in the same final model.

The discriminant function, on the basis of the input variables (e.g., CO_2, RSP, and nicotine levels), decides on whether a room should be classified as a smoking or a nonsmoking room (Table 3). The results of the discriminant analysis are then compared to actual room status.

Assuming that there is a significant difference between a nonsmoking and a smoking environment, then discriminant analysis should produce the following: (1) a discriminant model that is significant, and (2) a model which differentiates between smoking-observed and nonsmoking-observed rooms.

Software. A computer package by BMDP Statistical Software Inc. (#BMDP 7M) was used to generate this discriminant analysis. Standard statistical packages were used to produce the tables, graphs, and descriptive statistics.

RESULTS

Descriptive statistics

The final mix of building types surveyed is shown in Table 1. Since the establishment of designated smoking and nonsmoking areas may or may not be respected by occupants, and because even a smoking lounge may contain no smokers during our sampling period, it is not possible to classify areas as definitely smoking or nonsmoking. Instead, we can classify areas based on the smoking activity observed to be in place during the sampling period. For information, however, 20 rooms sampled were noted as designated smoking lounges. There were 254 nosmoking-observed and 331 smoking-observed data sets, giving a total of 585 data sets.

Figures 1 through 4 show frequency distributions for four parameters, divided into smoking-observed and nosmoking-observed groups. These frequency distributions illustrate the basic features of the raw data.

Table 2 displays the mean and standard deviation of, among others, the following variables: (1) RSP, (2) nicotine, (3) CO_2, (4) CO, and (5) room size. These statistics are shown for the overall data set, and are also categorized by room type (observed smoking activity), smoking or nonsmoking.

To explore in more detail the relationships between some of these factors, correlation coefficients were calculated between various parameters. Strong correlation exists between the following variables: RSP and smoking density ($r = 0.5180$, $p < 0.01$); nicotine and smoking density ($r = 0.7007$, $p < 0.01$); RSP and nicotine ($r = 0.7345$, $p < 0.01$).

Poorer correlations are calculated between the following variables: carbon monoxide and smoking density ($r = 0.1792$, $p < 0.01$); carbon dioxide and RSP ($r = 0.1763$, $p < 0.01$); carbon dioxide and nicotine ($r = 0.0841$, $p < 0.05$). It should be noted that the small p-values imply a nonzero correlation which does not mean a strong correlation. Correlations less than 0.2 should be viewed as relatively weak.

Table 1. Numbers of office types sampled

General Commercial Office Areas	340
Banking Offices	152
Cafeterias	62
Newspaper Offices	14
Institutional (church, hospital, correctional or educational)	17
Total	585

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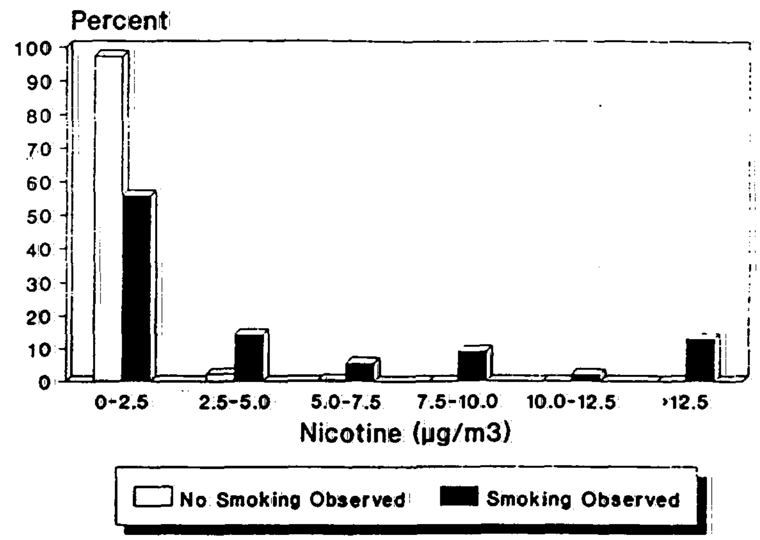

Fig. 1. Frequency distribution for nicotine measured in 585 office buildings with and without observed smoking.

Discriminant analysis

As indicated above, t-test results suggest that there is a significant difference between smoking and non-smoking rooms when considering the variables RSP ($p < 0.01$), Nicotine ($p < 0.01$), and CO ($p < 0.05$). However, there is serious overlap between the frequency distributions of smoking rooms and non-smoking rooms, particularly on the variables CO and RSP.

All factors in Table 2 were entered into the discriminant analysis (except number of cigarettes smoked). Smoker density was not entered into the

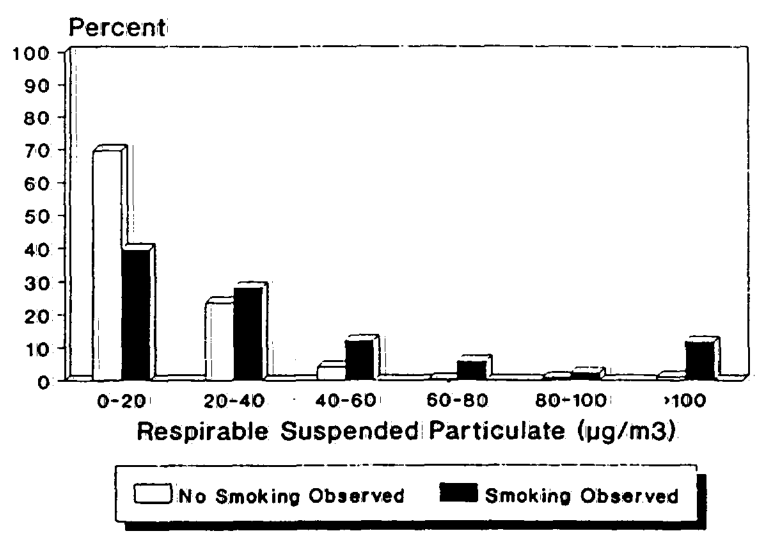

Fig. 2. Frequency distribution for respirable suspended particles measured in 585 office buildings with and without observed smoking.

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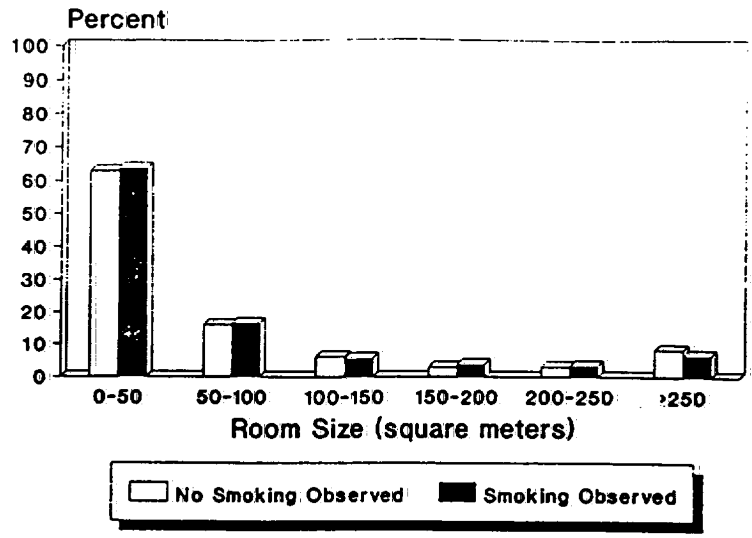

Fig. 3. Frequency distribution for room sizes of 585 office environments with and without observed smoking.

analysis since it is directly related to room type definitions— smoker density equal to zero is defined as a nonsmoking room and smoker density not equal to zero is defined as a smoking room. The discriminant analysis was significant and the variables RSP and nicotine were the only variables entered into the model. The discriminant analysis did not enter the variables CO and CO_2, nor the variables relating to room size and occupant density. All of these unentered variables do not improve the ability of the discriminant function to classify the rooms as smoking or nonsmoking.

Table 4 displays the ability of the selected model to predict room type properly. The selected model

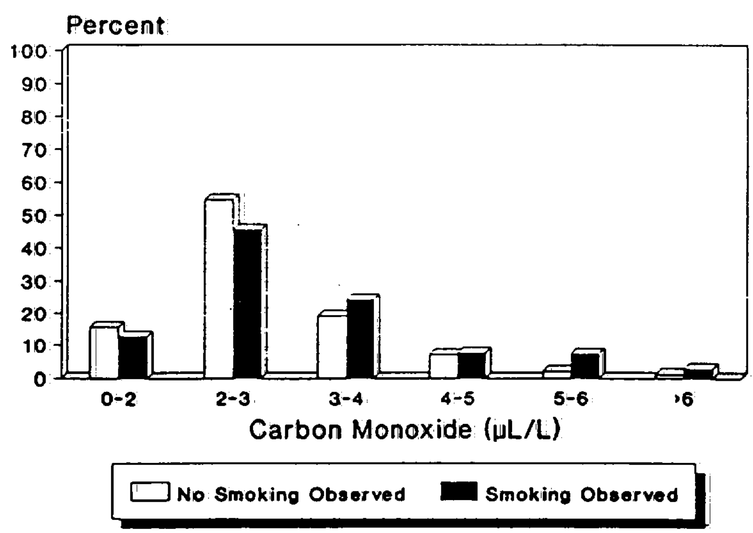

Fig. 4. Frequency distribution for carbon monoxide measured in 585 office environments with and without observed smoking.

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Table 2. The mean (Standard Deviation) of variables grouped by observed smoking activity.

Observed Smoking Activity	RSP* ($\mu g/m^3$)	Nicotine* ($\mu g/m^3$)	CO_2** ($\mu L/L$)	CO* ($\mu L/L$)	Room Size** (m^3)	# of People	# of Cigarettes Smoked
Non-Smoking	20 (17.6)	0.2 (0.8)	591 (159)	3.1 (0.90)	107 (231)	7.9 (15.7)	0.0 0.0
Smoking	46 (56.9)	6.7 (14.8)	595 (167)	3.4 (1.12)	83 (134)	7.9 (12.6)	5.9 (12.8)
Totals	35 (44.4)	3.8 (11.2)	593 (163)	3.29 (1.03)	93 (182)	7.9 (14.0)	---- ----

* Statistically significant difference between nonsmoking and smoking group.
** No statistically significant difference between nonsmoking and smoking group.

was able to classify properly 96.1% of the nonsmoking rooms as nonsmoking. However, only 41.4% of the smoking rooms were classified as smoking; 58.6% of the smoking rooms were classified as nonsmoking. Overall, 65.1 of the cases were properly classified.

Table 5 displays the mean and standard deviation of selected variables in the ETS data set. These variables are grouped according to observed status, either smoking or nonsmoking, and status provided by discriminant analysis. One possible combination, ob-

Table 3. Classification functions which determine model group membership, derived from discriminant analysis of the sample data.

Group	Constant	RSP	Nicotine
Nonsmoke	-0.8916	0.0202	-0.0557
Smoke	-1.2628	0.0284	-0.0269

Table 4. Classification of observations into smoking and nonsmoking groups by the discriminant analysis model based on sample results (the percentage of correct classifications is also shown).

Observed Status	Percent Correct	Model Status	
		Non Smoke number of sets	Smoke number of sets
Nonsmoke	96.1	244	10
Smoke	41.4	194	137
Total	65.1	438	147

Table 5. The mean (Standard Deviation) of selected variables shown for groups defined by their status as observed in the field during sampling and the discriminant analysis model based on sample results.

Observed Status	Model Status	RSP* $\mu g/m^3$	Nicotine** $\mu g/m^3$	CO_2* ($\mu L/L$)	CO* ($\mu L/L$)	Smoking Density** $cig/m^2.hr$
Nonsmoke	Nonsmoke	17.18 (9.5)	0.1 (0.6)	584 (153)	3.1 (0.8)	0.0 (0.0)
Smoke	Nonsmoke	19 (9.2)	0.9 (1.9)	566 (170)	3.3 (1.0)	0.075 (0.075)
Smoke	Smoke	85 (71.8)	14.8 (20.4)	636 (154)	3.6 (1.3)	0.30 (0.35)

*No statistically significant difference between Nonsmoke/Nonsmoke and Smoke/Nonsmoke. All other differences are statistically significant.
**Statistically significant differences between all groups.

served-status nonsmoking/model-status smoking, is not included since this combination only contains ten observations. The data show that for groups defined by observed-status nonsmoking/model-status nonsmoking and observed-status smoking/model-status nonsmoking, there is no statistically significant difference between group means for the variables CO, CO_2, and RSP. The group smoking-observed status/smoking-model status is significantly different when considering nicotine and smoker density. This difference is slight for the groups nonsmoking-observed status/nonsmoking-model status and smoking-observed status/nonsmoking-model status.

DISCUSSION

Discriminant analysis

The most significant results from the discriminant analysis are the following:

(1) RSP and nicotine contribute to the prediction of room type—smoking or nonsmoking.

(2) most (96.1%) nonsmoking rooms are classified as nonsmoking rooms, demonstrating very little evidence of ETS spillover from smoking areas, and

(3) a significant number (58.6%) of total smoking rooms are classified as nonsmoking rooms.

Table 5 suggests that smoking rooms can be separated as "light" or "heavy". The light-smoking rooms appear equivalent to nonsmoking rooms when considering, in a multivariate context, the important factors of RSP and nicotine. The heavy-smoking rooms do have elevated levels of nicotine and RSP. This indicates that there may be a rough working range of smoker density in which smoking activity does not seem to influence ETS levels significantly, as measured by RSP and nicotine.

This analysis goes part-way towards identifying what this range might be in that the mean of the light smoking range is 0.075 $cig/m^2 \cdot h$ and the median figure is 0.048 $cig/m^2 \cdot h$. There is considerable overlap, however, between the two types of smoking groups in Table 5. The median of the heavy-smoking rooms is 0.143 $cig/m^2 \cdot h$ with 20% of the heavy-smoking rooms below the median of the light-smoking rooms, and 13.4% of the light-smoking rooms above the median of the heavy-smoking rooms. This suggests that other variables which were not fully characterized in this work, such as outside air ventilation and/or air change rates, need to be considered in more detail when determining the impact of smoking in the room environment. One can see, however, that a realistic smoking density in properly ventilated rooms might be somewhere between 0.05 and 0.1 $cig/m^2 \cdot h$, or between 5 and 10 cigarettes per hour in a 100 m^2 room.

Other pertinent data

These 585 data sets reveal some other interesting information. If one examines the absolute levels of items of particular concern, such as nicotine, RSP

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and carbon monoxide, they show relatively conservative values. For instance, most of the RSP data in smoking-observed areas show levels well under that reported in Repace and Lowrey's early papers (1980, 1982); in general, about four times less than their mathematical model predicted for office environments of 200 $\mu g/m^3$ (Repace and Lowrey, 1987). In this model, they assumed an occupancy of 7.53 persons/100 m^2, one third of them smoking at an average rate of 2 cig/h (5.02 cig/100 $m^2 \cdot h$). In this study, in observed-smoking areas, mean RSP levels of 46.37 $\mu g/m^3$ were measured with a mean occupancy of 9.57 persons per 100 m^2, and an average observed-smoking rate of 7.14 cig/100 $m^2 \cdot h$. In fact, only eight data sets showed RSP values above this model value of 200 $\mu g/m^3$.

Our measurements of RSP and nicotine also tend to match levels reported in other recent work by Meisner et al. (1989) and Eatough et al. (1989a). Ogden et al. (1990) found ETS contributes approximately 50% to RSP which mirrors almost exactly our findings in that mean RSP levels in nosmoking-observed areas were approximately half those in smoking-observed areas.

There are statistically significant differences between smoking-observed and nonsmoking-observed areas for RSP, nicotine, and CO. While not statistically significant ($p \geq 0.10$), our data set contained smoking observed rooms with smaller sizes than nonsmoking rooms, suggesting that smokers in modern office environments may be confined to smaller rooms than nonsmokers. This possibility might justify further work, perhaps examining smokers/nonsmokers room sizes, separate from designated smoking lounges.

A clear relationship was observed between RSP and nicotine, which is not surprising in light of other studies. These studies were summarized by Eatough et al. (1989b), and show that nicotine/RSP ratios vary depending on overall ETS levels and tend towards that found in pure sidestream smoke at the highest levels of measured nicotine and RSP.

Although it is possible to identify two different groups (smoking and nonsmoking) by examining the carbon monoxide results, the distinction is not clear enough to characterize the relationship between smoking density and carbon monoxide concentrations. For instance, it was not possible to extrapolate properly the data to identify what smoking density might be associated with CO concentrations higher than the EPA ambient 24 h maximum of 9 $\mu L/L$, although it would appear to require a smoker density much greater than typical in a "discretionary smoking" office environment.

This shows that carbon monoxide is a poor indicator for ETS levels found in typical conditions. This is in contrast to Cain and Leaderer's work (1982) in experimental chambers which showed wide variations in carbon monoxide concentrations under different smoking conditions. Lower, more typical smoking rates, and larger spaces allowing for faster diffusion of CO may explain why this gas is not as good a predictor in normal offices as it is in experimental chambers.

Improved resolution for carbon monoxide (measured to +/-1 $\mu L/L$ in this study), and a lower detection limit for nicotine (set at 1.6 $\mu g/m^3$ for this study), and RSP (set at 10.0 $\mu g/m^3$) may be thought to allow for different conclusions to be drawn from the statistical analysis. However, a sensitivity analysis was performed with the discriminant analysis by setting different values for nicotine and RSP at the detection limits. For example, on a subsequent evaluation of the data with discriminant analysis, all nicotine and RSP values below the detection limit were set to zero. This produced little or no difference in the results obtained from the discriminant analysis. This strongly suggests that improved detection techniques would not change the results drawn from this study, including those conclusions concerning spillover from smoking areas.

Carbon dioxide is frequently used as an indicator of ventilation rates, and furthermore, since carbon dioxide levels are related to the number of occupants and the size of the space they occupy, these factors were also included in an attempt to establish a general ventilation status for each data set. No relationship was subsequently observed, however, between components of ETS and these factors as measured during these surveys. This does not mean that there is no relationship between total outdoor air intake for the building and overall ETS levels. But it means that local measurement of CO_2 needs to be interpreted carefully if it is to be used as an indicator of ventilation, and that it may show considerable spatial variations in a building, depending on local floor and air handling zone characteristics. This study did not examine in detail local room aspects of ventilation such as air change rates or airflow through local diffusers which may correlate much more strongly with local ETS levels. Alternatively, average levels of ETS throughout a building may also correlate with total outdoor air intake.

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CONCLUSIONS

These 585 measurements of some components of ETS and other related parameters sampled during 1989 suggest overall concentrations of ETS in typical office workspaces to be considerably lower than estimated ten years previously. Some parameters, such as carbon monoxide, appear to be only weakly related to smoking activity. Discriminant analysis shows that when "blindfolded" for presence or absence of smokers, in most cases realistic smoking levels do not significantly influence the aspects of air quality that were measured, and spillover from smoking areas into nonsmoking areas appears to be minimal. This work further reinforces the position the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) has taken on ETS in office buildings in ASHRAE Standard 62-89 (1989), in that acceptable air quality can be maintained in properly ventilated offices with a moderate amount of smoking, even without smoker segregation. These data help to further define the limits of moderate smoking.

Further work to achieve this goal should address room air exchange and ventilation rates, and their relation to ETS. This might best be achieved with the use of tracer gas instrumentation. This ventilation data when combined with ETS component measurements will give us a better understanding of the relationships between smoking and ventilation in modern offices. Other influencing factors may also include furnishing types and room size, which could be studied in more detail.

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Investigations of Ventilation, Smoking Activity, and Indoor Air Quality at Four Large Office Buildings

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ABSTRACT

Four large office buildings were investigated to assess relationships among ventilation rate, smoking activity, and indicators of indoor air quality (IAQ). Two types of heating, ventilating, and air-conditioning (HVAC) systems were examined: induction-constant air volume (CAV) and variable air volume (VAV). Two types of smoking policy were examined: unrestricted smoking and smoking restricted to lounges where air was exhausted directly to the outdoors. The four buildings represented all combinations of these four variables. One floor of each building was investigated, with measurements conducted during the summer when HVAC systems were not in the economizer mode, so they used minimal amounts of outside air. Inspections of HVAC systems indicated that they were adequately designed, operated, and maintained. Ventilation rates for office spaces were measured by standard procedures based on velocity traverses. During the tests, all four systems provided ventilation rates essentially conforming to ASHRAE 62-1989. *For the two buildings where smoking was unrestricted, 34% of occupants were smokers; the average smoking rate was 1.6 cigarettes per smoker per hour. Indicators of air quality and environmental tobacco smoke (ETS) were determined in offices and in outside and return airstreams. IAQ indicators included respirable suspended particles (RSP), formaldehyde, volatile organic compounds (VOCs), carbon dioxide (CO_2), and carbon monoxide (CO). ETS indicators included ultraviolet particulate matter (UVPM), fluorescent particulate matter (FPM), and nicotine. Results show that (1) within the context of the current ASHRAE standard, smoking activity had a measurable, although negligible, effect on contaminant levels in buildings where smoking was unrestricted; (2) restricting smoking to lounges eliminated nonsmoker exposure to ETS by preventing smoke from dispersing to adjacent areas; and (3) with the HVAC systems adequately designed, operated in accordance with the current ASHRAE standard, and properly maintained, all indicators were below applicable standards, regardless of smoking policy.*

INTRODUCTION

The last decade has seen increasing emphasis on issues relating to the quality of the indoor environment. This emphasis stems in great part from the relatively large proportion of time people spend there. Our IAQ research program has included surveys to assess exposures to airborne contaminants, in general, and ETS, in particular, in various indoor microenvironments such as offices (Oldaker et al. 1990).

ASHRAE 62-1989, Ventilation for Acceptable Indoor Air Quality (ASHRAE 1989), is a consensus standard that has two procedures for demonstrating acceptable IAQ. One procedure involves assessing whether ventilation rates conform with tabulated values; the other entails measuring contaminants to show that they are below specified levels. The work we report here is part of our ongoing research effort relative to IAQ in offices and has two general goals. Our scientific and technical goals were to assess practical implications of this ASHRAE standard and relationships between its two procedures. Our industrial hygiene goal was to obtain baseline information that can be used to address IAQ concerns in the future.

EXPERIMENTAL

Each of the four buildings was investigated during the working hours of one week in the summer of 1991. Smoking is unrestricted at Buildings A and B. At Buildings C and D, smoking is restricted to lounges where air is exhausted directly to the outdoors. None of the buildings had a history of IAQ problems. Tables 1a and 1b summarize characteristics of the buildings; Table 2 provides information on their HVAC systems. For Buildings A and C, private offices make up most of the floor area. For Buildings B and D, open office areas represent most of the floor area. In terms of age, size, occupancy, floor plan, and HVAC system, each pair of buildings was similar. At each building, one HVAC system and one floor served by that system were investigated. Selection of HVAC system and floor for testing at Buildings A and B was based upon maximal occupancy. The management of Buildings C and D selected HVAC systems and floors to be investigated that were representative of the buildings. At all buildings, occupants of floors tested were mostly clerical personnel and middle managers.

Contractors did all testing with the exception of (1) analyses for particulate substances and (2) monitoring of

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TABLE 1a
General Information on Four Buildings Investigated

Building	Year Occupied	Total number of floors	Total area of building (ft^2)	Number of Occupants (approx.)	Area of test (ft^2)	Number of Occupants in test space
A	1929	22	313,995	680	16,329	66
B	1982	16	471,000	1,400	27,801	114
C	1957	12	477,532	1,433	38,392	15
D	1990	10	570,000	1,000	32,000	54

TABLE 1b
General Information on Four Buildings Investigated

Building	Year Occupied	Total number of floors	Total area of building (m^2)	Number of Occupants (approx.)	Area of test (m^2)	Number of Occupants in test space
A	1929	22	29,170	680	1,516	66
B	1982	16	43,755	1,400	2,582	114
C	1957	12	44,362	1,433	3,566	15
D	1990	10	52,953	1,000	2,972	54

ventilation rates at Buildings B and D. On Mondays, HVAC systems were visually inspected to evaluate design, operation, and maintenance. Temperature and relative humidity were measured to assess thermal comfort. The fraction of outdoor air in the total air supplied to the test floors was quantified based on pitot tube measurements of volumetric flow rates. For VAV systems, the fraction of outdoor air and the total supply to each test floor were continuously monitored. Volumetric flow rates of air supplied by induction units of CAV systems were quantified based on use of empirical calibration curves relating volumetric flow rate to plenum pressure for given types of induction units.

On Tuesdays through Fridays, air samples were collected at randomly selected, occupied office spaces and in breathing zones. The numbers of offices sampled were 20 each for Buildings A and B, 14 for Building C, and 16 for Building D. For comparison with the samples collected in the offices, samples were collected daily from outside air and return air plenums.

RSP and UVPM were determined by the method described by Conner et al. (1990) with the inertial impactor separating at 2.5 μm. The method described by Ogden et al. (1990) was used to determine FPM. Nicotine and VOCs were determined by EPA Method IP-2A (Winberry et al. 1989a) and EPA Method IP-1B (Winberry et al. 1989b), respectively. Samples for RSP, UVPM, FPM, nicotine, and VOCs represented approximate seven-hour integrated averages. Formaldehyde was determined by NIOSH Method 3500 (NIOSH 1984) with samples collected for approximately one hour at the end of each day. CO_2 was determined with an NDIR analyzer. CO, sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were assessed with monitors employing electrochemical sensors. Ammonia (NH_3) and ozone (O_3) were screened with detector tubes. CO_2, CO, SO_2, NO_2, NH_3, and O_3 were sampled daily at three evenly spaced times with the average result used to represent an approximate seven-hour exposure.

A questionnaire was administered to occupants of Buildings A and B. The questionnaire was derived from those published by NIOSH (1987) and the Danish Building Research Institute (DBRI 1990). The questionnaire asked for information on symptoms experienced during the

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TABLE 2
HVAC Systems Serving Four Buildings

Building	Age of HVAC Systems (years)	Systems
A	35	39 fan-coil systems 1 induction systems (induction unit system has no return air capability)
B	10	2 cooling air handlers 2 heating air handlers 1 make-up air unit
C	36	6 peripheral systems 6 interior induction unit systems 26 air handlers
D	1	central fan section for AC/heating (1 for interior, 1 for exterior) 32 cooling zones 10 heating zones

previous three months including four strongly related to IAQ factors: (a) "itching, burning, or irritation of the eyes"; (b) "irritated, stuffy, or runny nose"; (c) "hoarse, dry throat"; and (d) "cough" (DBRI 1990). Smoking prevalence was quantified from responses to the questionnaire. Smoking rates were assessed by collecting and counting cigarette butts. Numbers of occupants were obtained from daily records of attendance.

RESULTS AND DISCUSSION

Inspections indicated that all systems were adequately designed, operated, and maintained. Relative humidities and temperatures for all test spaces ranged from 41% to 65% and 68°F to 78°F, respectively.

All four systems provided ventilation rates (in terms of cfm outdoor air per occupant) essentially conforming to *ASHRAE 62-1989*. For Buildings A and C, which have induction CAV technology, ventilation rates were 106 and 170 cfm (3.00 and 4.81 m^3/min)/occupant, respectively. Figures 1 and 2 show typical daily ventilation rates for Buildings B and D, respectively. At Building B, the average ventilation rate was 17 cfm (0.48 m^3/min)/occupant; ventilation rates varied from 12 to 21 cfm/occupant (0.33 to 0.59 m^3/min). Within the experimental constraints of this investigation, the -3 cfm (-0.08 m^3/min)/occupant average difference relative to the standard is considered negligible. The ventilation rate at Building D averaged 28 cfm (0.79 m^3/min)/occupant and ranged from 21 to 32 cfm (0.59 to 0.90 m^3/min)/occupant. The maximal CO_2 concentration

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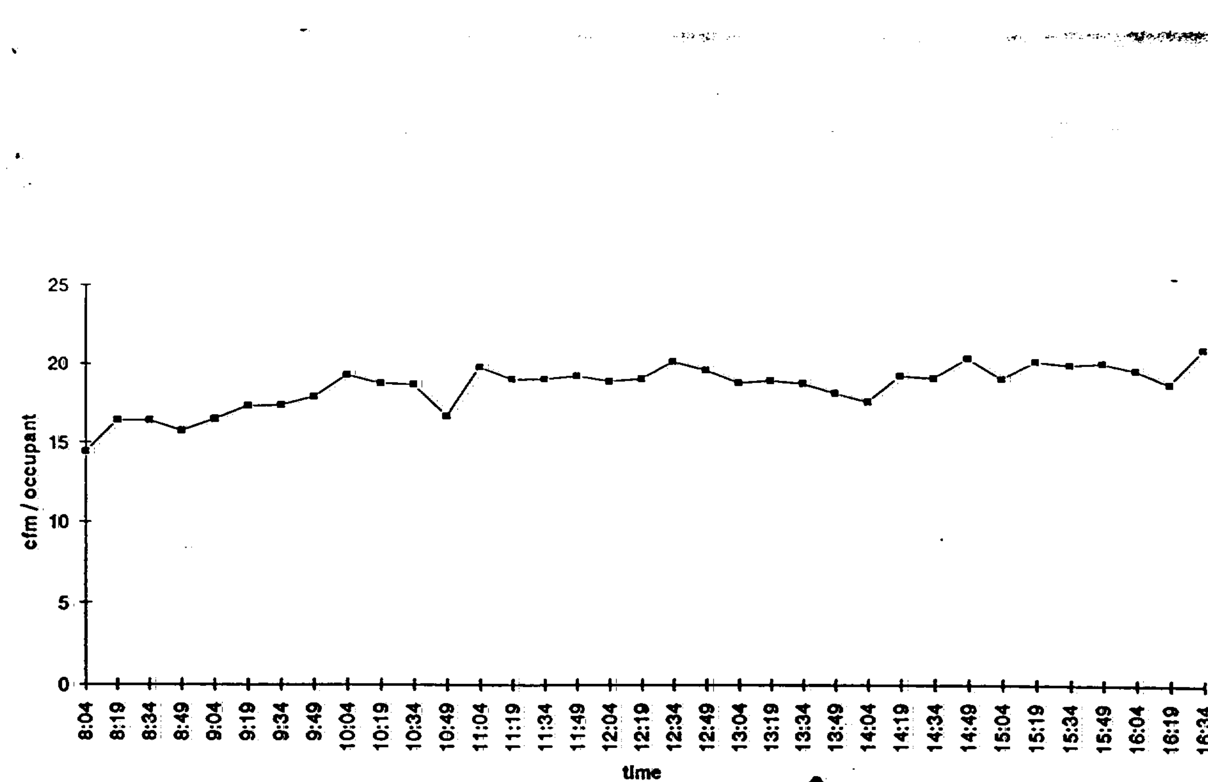

Figure 1 Ventilation rates for the test floor of Building ~~D~~ B *on July 10, 1991.*

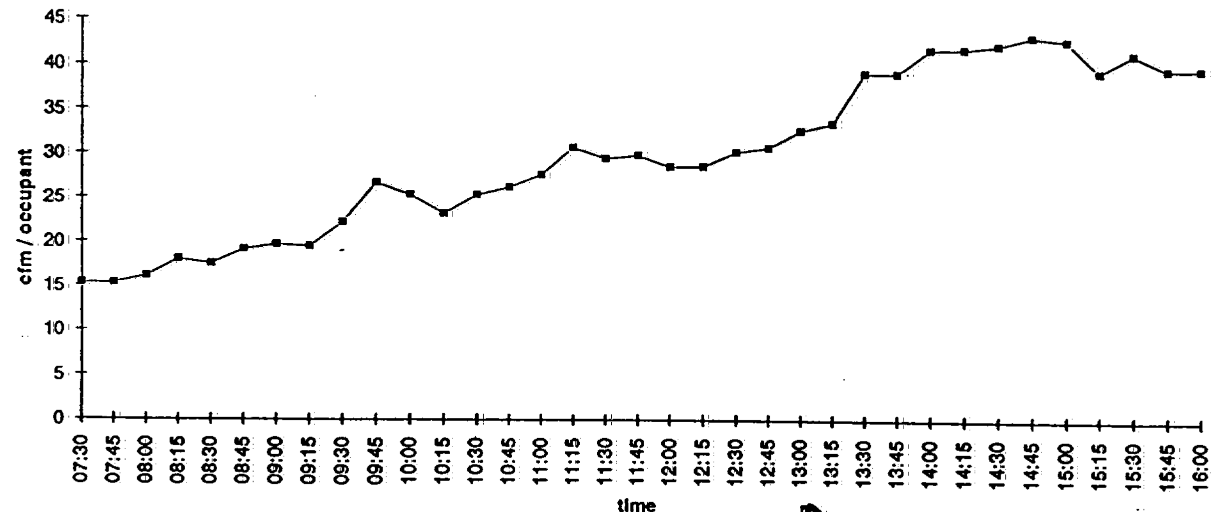

Figure 2 Ventilation rates for the test floor of Building D *on July 31, 1991.*

for the four buildings was 642 ppm, a result that supports the conclusion that ventilation was adequate.

At Buildings A and B, respectively, fractions of smokers as determined by questionnaire were 37.0% and 33.0%; the numbers of occupants responding were 65 and 121. The prevalence of smoking for both buildings, 34%, is substantially greater than the U.S. average, 28% (CDC 1991). Tables 3 and 4 present data on smoking activity for the two buildings. These data give an average smoking rate of 12.2 cigarettes per smoker per workday. Assuming that smoking occurred during the normal 7½-hour workday at the two buildings gives a smoking rate of 1.6 cigarettes per smoker per hour. This level of smoking activity is 18% less than the estimated U.S. average of two cigarettes per smoker per hour (NRC 1986).

To facilitate discussion, results from determinations of indicators of air quality are addressed relative to respective limits of detection (LODs) and limits of quantitation (LOQs), where the LOQ as used here is defined as three times the LOD. Of the 38 substances sampled, 24 were above limits of detection (LOD). Table 5 lists the 14 substances that were not detected and respective LODs. Of the 24 substances above LODs, four had medians below LODs for every building. (Because many data sets have substantial numbers of data below LODs, median values are used here for summarizing results. Medians, unlike avera-

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TABLE 3
Smoking Activity at the Test Floor of Building A

	DAY			
	Tues.	Wed.	Thurs.	Fri.
Number of cigarette butts	277	263	355	224
Number of occupants	53	51	52	50
Number of smokers	21	20	21	20
Number of nonsmokers	32	31	31	30

TABLE 4
Smoking Activity at the Test Floor of Building B

	DAY			
	Tues.	Wed.	Thurs.	Fri.
Number of cigarette butts	409	412	412	426
Number of occupants	110	108	108	108
Number of smokers	36	36	37	36
Number of nonsmokers	74	72	71	72

TABLE 5
Substances below Limits of Detection at All Buildings
(concentrations in $\mu g/m^3$ unless otherwise indicated)

Substance	Li. ᵗ of Detection
Ozone	0.01 ppm
Ammonia	0.1 ppm
Sulfur dioxide	1.5 ppm
Nitrogen dioxide	1.5 ppm
Carbon disulfide	0.8
Carbon tetrachloride	0.8
Chlorobenzene	0.8
Chloromethane	1.6
1,1-Dichloroethane	0.8
1,2-Dichloroethane	0.8
Isopropyl ether	0.8
Methyl *t*-butyl ether	0.8
1,1,2,2-Tetrachloroethane	0.8
Vinyl chloride	1.7

ges, do not depend on the convention used to assign a numerical value to data below LODs.) Chloroethane was quantified once at 15 $\mu g/m^3$ in Building C. Chloroform was detected in nine of the samples from Building B; the range of concentration was from 0.9 to 1.2 $\mu g/m^3$. Naphthalene was detected once at 1.3 $\mu g/m^3$ in the Building B and four times in Building C; for the latter, the range of concentration was from 0.9 to 1.4 $\mu g/m^3$. Finally, 1,1,2-trichloroethane was detected once at 1.5 $\mu g/m^3$ in Building D.

Table 6 presents medians and ranges for the remaining 20 substances found above LODs in offices at the buildings. Four have medians below LOQs; these are CO, formaldehyde, 4-methyl-2-pentanone, and styrene. CO is noteworthy because some researchers have used it as an indicator of ETS. Although the quality of the CO results here is limited because the monitor was accurate to ±2 ppm, the results are, nonetheless, in line with the position that CO is not a reliable ETS indicator because of its poor selectivity (Guerin et al. 1992). The highest level of CO, 3.0 ppm, was found at Building D where smoking was restricted to lounges exhausted directly to the outdoors. Much of this CO presumably came from outdoor sources, since the average concentration of CO in the outdoor air supplied to the offices was 2.0 ppm.

For purposes of discussion, substances above LOQs can be placed in two categories based upon whether or not they were found only indoors. (Having been addressed above, results for CO_2 are not addressed further here.) Four substances found only indoors were 1,4-dichlorobenzene, trichloroethene, 2-butanone, and nicotine. Nicotine was found only at Buildings A and B where unrestricted smoking occurred. This was expected since nicotine is an indicator of ETS. Interpreted strictly, however, the presence of airborne nicotine indicates only that smoking has occurred (Nelson et al. 1990).

Eleven substances were found both indoors and outdoors: RSP, UVPM, FPM, benzene, ethylbenzene, toluene, xylenes (all three isomers), methylene chloride, tetrachloroethene, 1,1,1-trichloroethane, and trichlorofluoromethane. These can be grouped as particulate species (RSP, UVPM, and FPM), arenes (benzene, toluene, ethylbenzene, and

TABLE 6
Summary of Results for Determinations of Substances in Office Spaces
at Concentrations Greater than Limits of Detection
(medians and ranges in $\mu g/m^3$ unless indicated otherwise; medians greater than LOQ indicated by asterisk [*])

	Building			
Substance	A	B	C	D
CO^2 (ppm)	500*	604*	484*	575*
	(433-575)	(558-620)	(333-542)	(533-642)
CO (ppm)	1.3 ppm	<1.0	2.0	2.4
	(0.7-2.0)	(<1.0-1.0)	(2.0-2.7)	(1.3-3.0)
Formaldehyde (ppm)	0.027	0.034	0.018	0.033
	(0.0085-0.041)	(0.024-0.047)	(0.012-0.020)	(0.030-0.040)
RSP	34*	30*	7	5
	(<7-74)	(<15-48)	(<9-20)	(<8-21)
UVPM	26*	16*	0.8	2*
	(<5-56)	(5-34)	(<1-2)	(0.4-4)
FPM	15*	14*	0.2	1*
	(2-30)	(3-31)	(<2-0.4)	(0.2-2)
Nicotine	1.8*	2.3*	<0.2	<0.1
	(0.1-11.7)	(0.3-9.9)	(<0.2)	(<0.1)
Benzene	2.1	4.8*	3.5*	1.7
	(0.8-4.4)	(3.7-7.6)	(2.7-4.9)	(1.4-3.6)
2-Butanone	<1.8	5.6*	<1.8	2.6*
	(<1.8-6.0)	(<2.2-14.0)	(<1.8-3.6)	(<1.6-3.4)
1,4-dichlorobenzene	<0.8	4.2*	<0.9	<0.8
	(<0.8-2.0)	(<0.9-5.3)	(<0.9)	(<0.8)

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TABLE 6 (Continued)
Summary of Results for Determinations of Substances in Office Spaces
at Concentrations Greater than Limits of Detection
(medians and ranges in $\mu g/m^3$ unless indicated otherwise; medians greater than LOQ indicated by asterisk [*])

Substance	Building A	B	C	D
Ethylbenzene	1.1	3.0*	1.6	1.6
	(0.9-2.2)	(<0.9-4.0)	(<0.8-3.6)	(1.4-3.0)
4-Methyl-2-pentanone	<1.8	1.7	<1.8	1.2
	(<1.8)	(<2.2-8.6)	(<1.8-3.3)	(0.9-2.1)
Methylene chloride	<0.8	<1.1	<0.9	8*
	(<0.8-7.1)	(<1.1-6.3)	(<0.9-1.2)	(<0.8-23.0)
Styrene	<0.9	1.9	<0.9	0.8
	(<0.9-1.3)	(1.6-2.5)	(<0.9)	(<0.8-1.0)
1,1,2,2-tetrachloroethylene	1.5	2.2	1.4	2.6*
	(<0.9-3.4)	(1.4-2.8)	(1.2-1.8)	(1.5-5.4)
Toluene	9.5*	19*	12*	21*
	(4.7-17)	(17-27)	(8.6-21)	(17-62)
1,1,1-trichloroethane	18*	50*	46*	29*
	(4.9-73)	(39-71)	(25-58)	(13-76)
Trichloroethene	2.8*	8.2*	2.9*	5.8*
	(<0.8-16)	(6.3-14)	(2.4-4.8)	(4.1-8.3)
Trichlorofluoromethane	1.2	1.2*	<0.9	5.2*
	(<0.8-1.8)	(<0.9-29.0)	(<0.9)	(3.9-7.1)
Xylenes	4.9*	12.0*	7.9*	6.0*
	(<0.8-9.1)	(2.6-16.0)	(5.8-16.0)	(4.7-11.0)

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xylenes), and chlorinated hydrocarbons (methylene chloride, tetrachloroethene, 1,1,1-trichloroethane, and trichlorofluoromethane).

Levels of RSP, UVPM, and FPM were higher for Buildings A and B than for Buildings C and D. For RSP, this difference reflects, in part, differences in outdoor levels of RSP. At Buildings B, C, and D, indoor levels of RSP were not statistically different from outdoor levels ($P > 0.05$). For Buildings C and D, median concentrations of RSP indoors and outdoors were the same. At Building B, the median outdoor level of RSP was 26 $\mu g/m^3$. RSP, because of its lack of selectivity and sensitivity, is a relatively poor indicator of particulate matter from ETS. Based on degree of selectivity and sensitivity, UVPM is better and FPM is the best of the three indicators of particles from ETS (Ogden et al. 1990). Based on FPM results, the estimated contribution of particles from smoking in Buildings A and B is about 15 $\mu g/m^3$.

The arenes are associated with products of combustion, solvents, and motor fuels. The presence of arenes both indoors and outdoors is expected, especially since all four buildings are located in urban areas. Indoor levels of arenes are at essentially the same magnitude for all four buildings.

The chlorinated hydrocarbons are associated with solvents, dry-cleaning operations and residues on clothing, and refrigerants (trichlorofluoromethane only).

Table 7 summarizes responses (by symptom) to the questionnaire for Buildings A and B. Response rates for the questionnaire were 87.9% at Building A and 91.7% at Building B. Response rates for symptoms are computed relative to the number of questionnaires returned. Rates for symptoms range from 1.7% (for "Cough" at Building A) to 19.0% (for "Itching, burning, or irritation of the eyes" at Building A). In general, rates at Building A are much greater than those at Building B.

Although the questionnaire was not designed to address acceptable IAQ per se, it can be used for this purpose by assuming that the converse of the criterion within *ASHRAE 62-1989* is applicable:

> The air can be considered acceptably free of annoying contaminants if less than 20% of a panel of at least 20 untrained observers deems the air to be objectionable under representative conditions of use and occupancy ... .

Application of this criterion to the individual response rates shown in Table 7 implies that IAQ was generally acceptable at both buildings.

At the four buildings, levels of all contaminants were within respective standards used for industrial hygiene applications. Relative to standards for VOCs, benzene reached the highest level. The maximal concentration of benzene, 7.6 $\mu g/m^3$, is more than four orders of magnitude below the ACGIH (eight-hour time weighted average) threshold limit value (TLV) of 32 mg/m^3 (ACGIH 1990). All levels of formaldehyde are below both federal and state standards as tabulated in Appendix C of *ASHRAE 62-1989*. The largest concentration of CO was 3.0 ppm; this value is below both the ACGIH TLV of 50 ppm and the eight-hour average of 9 ppm recommended in the ASHRAE standard.

TABLE 7
Summary of Responses to Categories of Symptoms

Symptom	Building A		Building B	
	%	Number	%	Number
Itching, burning, or irritation of the eyes	19.0	11	7.2	8
Irritated, stuffy, or runny nose	15.5	9	8.1	9
Hoarse, dry throat	10.3	6	5.4	6
Cough	1.7	1	2.7	3

In addition, levels of RSP are below levels suggested by the ASHRAE standard. The greatest concentration of RSP (measured in the private office of a smoker) was 74 $\mu g/m^3$. Based upon averaging time, the most appropriate standard for interpreting this approximate seven-hour exposure is the 24-hour average, primary National Ambient Air Quality Standard of 150 $\mu g/m^3$.

Results indicate that the smoking lounges at Buildings C and D were effective at preventing dispersal of smoke to offices on the same floor. The tobacco-selective contaminants, nicotine, UVPM, and FPM, were either below the LOD (as was the case for nicotine) or at background levels determined in the outdoor air. These results are expected based upon two facts. First, air from both lounges is exhausted from each building rather than being recirculated. Second, results from assessments of ventilation with smoke tubes showed that the lounges were at a pressure slightly negative relative to adjacent occupied spaces on the floor.

CONCLUSIONS

Investigations of indoor air quality in two buildings where smoking was unrestricted and two buildings where smoking was restricted to lounges showed:

1. In these buildings, with the HVAC systems adequately designed, operated in accordance with the current ASHRAE standard, and properly maintained, all indicators were well within applicable standards, regardless of the smoking policy.
2. Smoking activity had a negligible effect, in general, on contaminant levels in buildings where smoking was unrestricted. Although levels of some contaminants were higher than those in the buildings where smoking was restricted, all contaminants measured were within applicable standards.
3. Restricting smoking to lounges eliminated nonsmoker exposure to ETS by preventing smoke from dispersing to adjacent areas. No tobacco-selective contaminants were indicated in office spaces on the same floor tested.
4. Smoking was associated with increased indoor levels of respirable suspended particles (RSP), but these increases were small with ventilation rates conforming to the current *ASHRAE 62-1989*. RSP levels were all within accepted standards in all four buildings during all tests.

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THE EFFECTS OF ALTERNATIVE SMOKING POLICIES ON INDOOR AIR QUALITY IN 27 OFFICE BUILDINGS

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Abstract – The effect of alternative smoking policies, which prohibited or restricted smoking, on indoor air quality was studied in 27 air-conditioned office buildings. Carbon monoxide, carbon dioxide, respirable particulates, formaldehyde, ultraviolet particulate matter (u.v.PM), nicotine, air temperature, relative humidity and illumination were measured at eight sample sites in each building. Smoking policy had no effect on carbon monoxide, carbon dioxide, relative humidity, formaldehyde, air temperature or illumination for open office areas. It did have an effect on levels of respirable suspended particulates, ultraviolet particulate matter and nicotine.

INTRODUCTION

TOBACCO smoking indoors can be a source of a number of indoor air contaminants, especially particulate matter (HODGSON, 1989) and gaseous products (GODISH, 1990). Burning tobacco releases into the air several thousand pollutants, of which about 400 have been quantified (U.S. SURGEON GENERAL, 1986; EISENBERG, 1992). Environmental tobacco smoke (ETS) is an aerosol comprising vapour and particulate phases, both of which contain many organic and inorganic compounds (EATOUGH *et al.*, 1988; GUERIN *et al.*, 1992). ETS is a combination of sidestream smoke from the burning cigarette and exhaled mainstream smoke from the smoker (GUERIN *et al.*, 1987; REASOR, 1987). The exposure of non-smokers to ETS or to 'second-hand smoke' is termed 'involuntary smoking' or 'passive smoking' (DOUVILLE, 1990). Passive smoking has been identified as a risk factor for chronic health problems, such as lung cancer and cardiovascular disease (OFFICE OF TECHNOLOGY ASSESSMENT, 1986), and for the sick building syndrome (ROBERTSON *et al.*, 1988).

In an attempt to improve indoor air quality in the workplace and to reduce risks of the sick building syndrome (SBS) among workers, 42% of U.S. office buildings now prohibit tobacco smoking (INTERNATIONAL FACILITIES MANAGEMENT ASSOCIATION, 1992). However, up to one-third of workers in U.S. office buildings are smokers (HEDGE *et al.*, 1991; OLDAKER *et al.*, 1992), and to balance the needs of non-smokers and smokers many companies have chosen to implement spatially restrictive smoking policies rather than to prohibit smoking. Several such policies are operated currently. Sometimes smoking is confined to areas with a ventilation system which is separate from that for the non-smoking offices. In some buildings smoking is simply confined to certain areas without any localized air treatment before it is returned to the ventilation system, in others localized air filtration removes the bulk of the ETS pollutants before the air returns to the ventilation system, and rather than requiring smokers to

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congregate in specific places, some companies restrict smoking to individual enclosed offices or to open-plan partitioned cubicle workstations.

Spatially restrictive smoking policies appear to have little impact on indoor air quality. STERLING et al. (1987) compared two similar office floors, one where smoking was unrestricted and one where it was restricted to a designated area. Nicotine concentrations were elevated on the unrestricted smoking floor (average of $4.9\ \mu g\ m^{-3}$), but there were no significant differences in the concentrations of carbon monoxide, carbon dioxide or respirable particulates. OLDAKER et al. (1992) compared two buildings where smoking was restricted to lounges with two buildings where smoking was unrestricted. They found that concentrations of respirable particulates were higher in the unrestricted smoking buildings, otherwise smoking activity had a negligible effect on indoor pollutants.

A field experiment was conducted to test the effect of five smoking policies (prohibition and various forms of spatial restriction) on indoor air quality in 27 air-conditioned office buildings.

METHODS

Smoking policies

Five smoking policies were selected for investigation: smoking prohibited (SP); smoking restricted to rooms ventilated by a separate ventilation system (RSV); smoking restricted to rooms with local electrostatic air filtration units (RF); smoking restricted to areas with no local air treatment (RNT); and smoking restricted to enclosed offices and open-plan partitioned cubicle workstations (RWS).

Office buildings

Twenty-seven air-conditioned buildings with either variable air or constant air volume ventilation systems were selected on the basis of their smoking policy, office layout, nature of office activities and location (Table 1). The buildings were occupied by 17 organizations performing typical office work (e.g. insurance, finance, sales and marketing). Fifteen of these, occupying 25 of the 27 buildings, were private companies. One building was occupied by a federal agency, and one by a municipality. The investigators were blind to the indoor air quality status of the buildings, apart from one building where an indoor air quality problem was suspected prior to study. All the buildings were in the Eastern or mid-Western U.S.A.: Alabama, Georgia, Illinois, Indiana, Kentucky, Massachusetts, Michigan, Minnesota, New York State, Ohio and Virginia.

Selection of indoor air quality survey sites

Except in building G indoor air quality in each building was sampled in the winter spring season over two consecutive workdays; building G was sampled for 1 day, because a census sample of workers had been achieved. On each day two sites were sampled in the morning and two more in the afternoon. The total number of sites surveyed for each smoking policy is shown in Table 2.

Indoor air quality sampling

All sampling was conducted during normal office hours and on full working days. Where possible, sample sites were chosen in the most densely occupied office areas with

TABLE 1. SUMMARY CHARACTERISTICS OF THE OFFICE BUILDINGS

Policy	Building	Site	Age (years)	Total area (m^2)	No. of floors	HVAC*	Design air change rates	Humidification
Smoking prohibited (SP)	A	City, busy road	50 and 30	61 316	10	VAV	na	spray
Six buildings	B	Office park	6	13 471	4	VAV	8·9 h^{-1}	evaporative
	C	City, busy road	10	20 903	13	CAV	na	none
	D	City, busy road	23	25 084	19	CAV	3·8 h^{-1}	none
	E	Office park	16	5295	3	CAV	7.5 h^{-1}	none
	F	City, busy road	23	25 084	19	CAV	3·8 h^{-1}	none
Restricted smoking areas with separate ventilation (RSV)	G	City, busy road	1	48 774	5	VAV	9 h^{-1}	none
	H	City, quiet road	34	18 023	3	CAV	9 h^{-1}	none
	I	City, busy road	68	33 445	18	CAV	na	none
Four buildings	J	Office park	12	50 911	6	VAV	na	steam
Restricted smoking areas with room air filtration (RF)	K	Office park	5	6968	2	VAV	na	none
	L	Office park	5	6968	2	VAV	na	none
	M	Office park	1	18 581	5	VAV	na	none
Six buildings	N	City, busy road	20	55 742	40	CAV	na	none
	O	Office park	2	7804	4	VAV	na	none
	P	City, busy road	27	87 143	30	CAV	na	none
Restricted smoking areas with no treatment (RNT)	Q	City, busy road	8	92 903	40	VAV	na	none
	R	City, busy road	15	5110	2	VAV	na	none
Five buildings	S	Office park	14	10 219	4	VAV	8·9 h^{-1}	none
	T	City, busy road	20	108 975	51	CAV	na	none
	U	Office park	21	15 236	4	CAV	6-7 h^{-1}	none
Smoking restricted to work stations (RWS)	V	Greenfield	1	55 742	7	CAV	8 h^{-1}	steam
	W	Office park	4	38 090	4	VAV	na	steam
Six buildings	X	City, busy road	24	14 400	3	CAV	6·8 h^{-1}	steam
	Y	City, busy road	8	34 839	4	CAV	6·8 h^{-1}	steam
	Z	City, busy road	17	16 258	14	VAV	na	none
	AA	City, busy road	13	29 729	19	CAV	na	evaporative

*CAV = constant air volume ventilation system; VAV = variable air volume ventilation system.
na = information not available.

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buildings B, D, F, H, J, K, L, M, N, O, R, S, V, W, X, Y, Z and AA the CO monitor used had a detection range from 0 to 50 ppm $\pm$ 1% full-scale accuracy, with a detection limit of 0.5 ppm. In the other buildings, the CO monitor used had a detection range from 0 to 10 ppm $\pm$ 1% full-scale accuracy, with a detection limit of 0.1 ppm. The instruments were zeroed as required and on each sampling day they were calibrated morning, early afternoon and at the end of the day with a certified span gas of 30 ppm CO in air.

Carbon dioxide (CO_2). CO_2 often may be an indicator of ventilation rate. Levels were measured with a portable non-dispersive i.r. absorption gas analyser with a measurement range of 50-9950 ppm ($\pm$0.01%, 50 ppm detection limit). The monitor was calibrated using 99.99% dry nitrogen as a zero gas and a span gas of 1000 ppm CO_2 in nitrogen in the morning, early in the afternoon and at the end of the day (this instrument proved to be sensitive to orientation, and vertical or horizontal orientation produced different readings: all calibration and measurements were performed in the vertical position). Once the monitor's reading was stabilized ($\sim$10 s), a 1-min average concentration was read.

Respirable suspended particulates ($RSP_{3.5}$). A piezoelectric microbalance (3.5 μm impactor) was used to measure $RSP_{3.5}$ (the instrument was factory-calibrated with dilute welding fumes, with a sensitivity of 180 mg Hz^{-1}). The measurement range of the instrument was 0.01 3.5 mg m^{-3}. The sensor was cleaned every four measurements, and the impactor and precipitator needle were cleaned prior to sampling in each building. For each measure, air was sampled for 120 s (10 μg m^{-3} detection limit). $RSP_{3.5}$ underestimates the respirable particulates from ETS by 10 15% (INGEBRETHSEN *et al.*, 1988).

Illuminance. Horizontal illuminance was measured with a digital illuminance meter with a measurement range of 0.01 to 99 900 lux $\pm$ 2%. Areas adjacent to windows were avoided because these were not representative of the illuminance levels for the majority of the workspaces sampled.

Temperature (°C) *and relative humidity* (*RH*). Temperature and RH were measured with a digital hygro-thermometer. For temperature the measurement range was 0.0 80.0°C with 0.1°C resolution and an accuracy to $\pm$0.1°C; for RH it was 10 95% RH, with 0.1% resolution and an accuracy of $\pm$2%.

Statistical design and analysis of the indoor environmental data

The physical environment data were analysed as a split unit design, with smoking policy as the whole unit treatment factor and time-of-day (morning or afternoon samples) and smoking designation of an area (smoking or non-smoking) as the sub-unit treatment factors. The building was the experimental unit for smoking policy. The effect of smoking policy was tested using the pooled variation in mean response among the buildings within each policy as the error term. The locations at which the pollutant and environmental measures were taken within each building was the experimental unit for the time-of-day smoking designation treatment combinations. Effects of time-of-day, smoking designation, interactions between these factors and interactions of

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these factors with smoking policy were tested with the variation among locations in buildings as the error term. All interactions were tested at a liberal $P=0.1$, whereas main effects and contrasts were evaluated at $P=0.05$. The design was unbalanced because of unequal numbers of buildings for each policy and unequal numbers of smoking and/or non-smoking sites in a building as determined by the smoking policy. Consequently, mean values and standard errors given in the results are least-squares estimates for unbalanced models (i.e. means of the effects in a model which would be expected if the design were balanced). When the effect of policy was significant for a pollutant, comparisons among the smoking policies were made using the following set of orthogonal contrasts: RSV vs RF (separate ventilation vs filtration); RNT vs average of RSV and RF (dilution from smoking areas vs some form of air cleaning); SP vs average of RNT, RSV and RF (no smoking vs some form of spatial restriction of smoking); RWS vs average of SP, RNT, RSV and RF (dilution of dispersed sources vs spatial restriction or no sources). An orthogonal set is the most concise way of summarizing the policy differences using independent pieces of information (SNEDECOR and COCHRAN, 1989).

To assess properly the main effect of smoking area designation and the interaction between smoking area designation and policy, separate analyses using only the data from the spatially restricted smoking policies (RSV, RF, RNT) were performed. When the interaction between policy and smoking designation was significant for a pollutant, comparison of measures between smoking and non-smoking areas were made for each spatially restricted smoking policy (RSV, RF, RNT). Where appropriate, these comparisons were made using unequal variance-independent samples t-tests.

Partial correlations among the environmental measures were calculated to assess how tightly coupled the concentrations of the pollutants associated with tobacco smoke were, after accounting for the variation in the measurements due to the design variables. The statistical analysis of the concentrations of CO, CO_2 and formaldehyde were performed on the natural log scale, because the variance varied with the mean, indicating that these data were log-normal. Because there were zero readings on the concentration scale for CO and formaldehyde, for computational purposes, a value of 10^{-4} was added to each CO reading and zero readings for formaldehyde concentration were replaced with the detection limit of the method (0.018 ppm). Statistical analyses of the physical environment data were performed using multivariate statistical analysis software (SAS v.5.18).

RESULTS

The gravimetric method used to measure $RSP_{2.5}$ was found to be unreliable, with 26 of 27 field blanks showing some discrepancy from their initial mass: six with negative ss, 20 with positive mass. u.v.PM was not significantly correlated with gravimetric $RSP_{2.5}$, even though the u.v.PM samples were derived from these RSP samples. Although statistical analysis of gravimetric $RSP_{2.5}$ had been reported for 18 of these buildings in an interim paper (HEDGE *et al.*, 1991), the results from the 27 buildings were considered unreliable and were not further analysed.

The average percentage of current smokers was comparable for each policy (SP = 19%, range 13–26%; RSV = 20%, range 16–25%; RF = 22%, range 15–30%; RNT = 16%, range 7–29%; RWS = 15%, range 10–20%). Estimates of the total

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number of cigarettes smoked during the workday by the smokers surveyed in the RSV, RF, RNT and RWS policies, and the total number of person hours spent in smoking areas in the RSV, RF and RNT policies were calculated from the questionnaire survey data. No significant differences among the policies in which smoking was allowed were found either for the mean number of cigarettes smoked daily [RWS (mean = 345, SE = 55), RNT (mean = 186, SE = 67), RSV (mean = 162, SE = 67), RF (mean = 191, SE = 55)], or for the total number of person hours spent in smoking areas of the spatially restricted policies [RSV (mean = 26.25, SE = 19.55), RF (mean = 57.63, SE = 15.99), RNT (mean = 18.75, SE = 22.57)] by the smokers surveyed.

Smoking policy had a consistent effect on indoor air quality for the pollutants measured, although the concentrations of pollutants generally were low. The overall effects of smoking policy on indoor air quality were tested using the mean values of the pollutants and of the environmental measures for each policy, regardless of the smoking designation of survey sites within buildings or of the time-of-day (Table 3). To assess the effects of smoking areas and of non-smoking areas on pollutants and environmental measures, the mean values for the smoking and for the non-smoking areas of the spatially restricted policies were used (Table 4). The effects of smoking policy on indoor air quality in the non-smoking office areas of the SP, RSV, RF and RNT policies and in the office areas of the RWS policy were tested using the means values of the pollutants and environmental measures for only the office areas for each policy (Table 5).

Formaldehyde concentrations generally were very low in the study buildings, although building AA had relatively high concentrations (range 0.027–0.052 ppm). There was no effect of smoking policy on formaldehyde, although there was an interaction of smoking policy with survey site ($F_{12,150} = 3.34$, $P = 0.0003$): formaldehyde concentrations were higher in smoking areas than non-smoking areas in the buildings where spatially restricted smoking policies applied, whereas there were no differences among the sites for the SP and RWS policies. To test the effects of smoking area designation on formaldehyde concentrations only the data from the areas of spatially restricted smoking policies (RSV, RF and RNT) were analysed. There was an interaction of smoking policy with smoking designation of the site ($F_{2,88} = 3.56$, $P = 0.0326$). There was no difference in formaldehyde concentrations between smoking and non-smoking areas for the RSV policy; the formaldehyde concentrations in smoking areas were double those in the non-smoking buildings for the RF policy; and the highest formaldehyde concentrations in the non-smoking and smoking areas were found for the RNT policy (Table 4). For only the buildings with spatially restricted policies, there was a marginal effect of smoking policy ($F_{2,12} = 3.75$, $P = 0.0543$), resulting from a difference between the RNT and the average of the RSV and RF policies ($F_{1,12} = 7.44$, $P = 0.0184$). When only the office area sites were analysed for all five policies, there was no significant effect of smoking policy on formaldehyde concentrations (Table 5), but the orthogonal contrasts showed that there was a significant difference between the RNT and the average of the RSV and RF policies ($F_{1,22} = 5.23$, $P = 0.0321$). After accounting for the variation in concentrations due to the design variables (policy, site and time-of-day) formaldehyde was positively correlated with CO ($r = 0.28$, $P = 0.0005$) and with CO_2 ($r = 0.26$, $P = 0.0013$).

There was no effect of smoking policy on CO, although there was an interaction of smoking policy with site for CO ($F_{12,150} = 1.96$, $P = 0.0320$). For the spatially restricted

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TABLE 3. INDOOR ENVIRONMENT CONDITIONS FOR EACH SMOKING POLICY* (LEAST-SQUARES MEANS AND STANDARD ERRORS)

	SP	RSV	RF	RNT	RWS
CO (ppm)†	0.1 [0.00, 0.5]	0.7 [0.1, 5.2]	0.4 [0.1, 2.1]	0.6 [0.1, 3.3]	0.1 [0.0, 0.5]
CO_2 (ppm)†	561 [478, 657]	600 [494, 729]	674 [575, 791]	634 [533, 755]	573 [488, 671]
Formaldehyde (ppm)†	0.01 [0.008, 0.028]	0.02 [0.009, 0.046]	0.02 [0.013, 0.047]	0.05 [0.025, 0.103]	0.01 [0.008, 0.028]
RSP ($\mu g\ m^{-3}$)	29.3 ± 9.3	63.1 ± 11.4	90.4 ± 9.3	65.7 ± 10.2	22.6 ± 9.3
u.v.PM ($\mu g\ m^{-3}$)	0.2 ± 22.9	44.1 ± 28.1	114.9 ± 22.9	19.8 ± 25.1	10.2 ± 22.9
Nicotine ($\mu g\ m^{-3}$)		6.90 ± 3.0‡	20.8 ± 2.4	3.8 ± 2.6	2.4 ± 2.4
Temperature (°C)	23.9 ± 0.4	24.3 ± 0.5	23.9 ± 0.4	23.7 ± 0.4	23.6 ± 0.4
RH (%)	29.7 ± 3.5	35.1 ± 4.3	33.4 ± 3.5	28.3 ± 3.9	35.9 ± 3.5
Illuminance (lux)	472.4 ± 62.4	513.3 ± 75.3	507.9 ± 62.4	586.4 ± 67.8	564.9 ± 62.4

*SP smoking prohibited; RSV smoking retricted to separately ventilated areas; RF—smoking restricted to rooms with local air filtration; RNT—smoking restricted to rooms with no additional air treatment; RWS smoking restricted to work stations.

†Least-squares means back-transformed from natural logarithm scale; back-transformed 95% confidence limits shown in square brackets.

‡Nicotine measured only in separately ventilated smoking areas.

TABLE 4. INDOOR ENVIRONMENT CONDITIONS FOR NON-SMOKING AND SMOKING AREAS OF SPATIALLY RESTRICTED SMOKING POLICIES* (LEAST-SQUARES MEANS AND STANDARD ERRORS)

	RSV Non-smoking	RSV Smoking	RF Non-smoking	RF Smoking	RNT Non-smoking	RNT Smoking
CO (ppm)†	0.3 [0.2, 0.7]	1.5 [0.7, 3.4]	0.2 [0.1, 0.3]	1.2 [0.7, 2.1]	0.4 [0.2, 0.8]	1.0 [0.4, 2.2]
CO_2 (ppm)†	573 [523, 627]	635 [571, 707]	673 [626, 723]	676 [625, 732]	597 [550, 647]	698 [624, 781]
Formaldehyde (ppm)†	0.02 [0.013, 0.024]	0.02 [0.017, 0.034]	0.02 [0.014, 0.022]	0.04 [0.030, 0.049]	0.05 [0.038, 0.063]	0.06 [0.039, 0.079]
RSP ($\mu g\ m^{-3}$)	33.6 ± 11.9	109.8 ± 14.0	37.0 ± 9.4	157.3 ± 10.3	38.9 ± 10.5	117.4 ± 14.7
u.v.PM ($\mu g\ m^{-3}$)	3.2 ± 23.1	109.1 ± 27.3	14.5 ± 18.3	236.6 ± 20.0	9.3 ± 20.5	39.2 ± 28.6
Nicotine ($\mu g\ m^{-3}$)		8.2 ± 5.1‡	0.9 ± 3.3	44.2 ± 3.6	0.3 ± 3.8	10.3 ± 5.2
Temperature (°C)	24.4 ± 0.2	24.4 ± 0.2	24.0 ± 0.2	23.8 ± 0.2	23.7 ± 0.2	23.8 ± 0.2
RH (%)	34.6 ± 1.3	35.4 ± 1.5	33.2 ± 1.0	33.6 ± 1.1	28.2 ± 1.2	28.7 ± 1.6
Illuminance (lux)	544.5 ± 48.4	488.5 ± 57.0	515.4 ± 38.7	503.6 ± 42.0	648.8 ± 43.0	474.5 ± 60.3

*SP—smoking prohibited; RSV—smoking restricted to separately ventilated areas; RF—smoking restricted to rooms with local air filtration; RNT—smoking restricted to rooms with no additional air treatment; RWS—smoking restricted to work stations.

†Least squares means back-transformed from natural logarithm scale; back-transformed 95% confidence limits shown in square brackets.

‡Nicotine measured only in separately ventilated smoking areas.

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TABLE 5. INDOOR ENVIRONMENT CONDITIONS FOR OPEN OFFICE AREAS OF EACH SMOKING POLICY* (LEAST-SQUARES MEANS AND STANDARD ERRORS)

	SP	RSV	RF	RNT	RWS
CO (ppm)†	0.1	0.3	0.2	0.4	0.1
	[0.0, 0.6]	[0.0, 3.2]	[0.0, 1.1]	[0.1, 3.2]	[0.0, 0.6]
CO_2 (ppm)†	561	573	673	598	573
	[483, 651]	[477, 687]	[580, 781]	[508, 704]	[493, 664]
Formaldehyde (ppm)†	0.01	0.02	0.02	0.05	0.01
	[0.007, 0.029]	[0.008, 0.041]	[0.009, 0.033]	[0.023, 0.100]	[0.007, 0.029]
RSP ($\mu g\ m^{-3}$)	29.3 ± 5.1	33.6 ± 6.3	36.1 ± 5.1	43.9 ± 5.6	22.6 ± 5.1
u.v.PM ($\mu g\ m^{-3}$)	0.2 ± 2.2	3.2 ± 2.7	11.0 ± 2.2	5.8 ± 2.4	10.2 ± 2.2
Nicotine ($\mu g\ m^{-3}$)	--	--	0.4 ± 0.7	0.7 ± 0.8	2.4 ± 0.5
Temperature (°C)	23.9 ± 0.4	24.4 ± 0.5	24.0 ± 0.4	23.7 ± 0.4	23.6 ± 0.4
RH (%)	29.7 ± 3.6	34.6 ± 4.4	33.3 ± 3.6	28.4 ± 3.9	35.9 ± 3.6
Illuminance (lux)	472.4 ± 62.4	544.5 ± 75.3	518.6 ± 62.4	629.5 ± 67.8	564.9 ± 62.4

*SP—smoking prohibited; RSV—smoking restricted to separately ventilated areas; RF—smoking restricted to rooms with local air filtration; RNT—smoking restricted to rooms with no additional air treatment; RWS—smoking restricted to work stations.

†Least-squares means back-transformed from natural logarithm scale; back-transformed 95% confidence limits shown in square brackets.

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policies. CO concentrations were higher in smoking areas than in non-smoking areas ($F_{1,88}=26.40$; $P=0.0001$; Table 4). CO concentrations were not significantly different among smoking policies for the office areas (Table 5).

There was a significant overall effect of smoking policy on u.v.PM ($F_{4,22}=4.01$, $P=0.0136$) and concentrations were higher in the areas applying restricted policies than in those applying the SP policy (Table 3). There was an interaction of smoking policy with site for u.v.PM ($F_{12,150}=6.99$, $P=0.0001$). Analysis of the data for the spatially restricted smoking policies showed that u.v.PM concentrations were higher in smoking areas than in non-smoking areas, although the difference varied among the smoking policies and was greatest for the RF policy ($F_{2,88}=9.55$; $P=0.0002$; Table 4). Comparisons of only the office areas among the five smoking policies also showed a policy effect ($F_{4,22}=4.08$, $P=0.00127$; Table 5), and the following contrasts were significant: SP vs average of RSV, RF and RNT ($F_{1,22}=5.97$, $P=0.0230$); and RWS vs average of SP, RSV, RF and RNT ($F_{1,22}=4.10$, $P=0.0552$). u.v.PM was positively correlated with $RSP_{3.5}$ ($r=0.45$, $P=0.0001$), after accounting for the variation due to the design variables.

Smoking policy affected $RSP_{3.5}$ ($F_{4,22}=8.84$, $P=0.0007$), because of the significant difference between the SP buildings and all the buildings with restricted policies ($F_{1,22}=15.53$, $P=0.0001$); and the buildings designated RWS and those applying other restricted smoking policies ($F_{1,22}=13.80$; $P=0.0012$; Table 3). For the buildings applying spatially restricted smoking policies $RSP_{3.5}$ was higher in smoking areas than in non-smoking areas (Table 3) ($F_{1,88}=85.37$, $P=0.0001$). There was a difference in $RSP_{3.5}$ among policies for the office areas (Table 4): $RSP_{3.5}$ was lower in the RWS policy than other policies ($F_{1,22}=5.06$, $P=0.0348$).

Nicotine concentrations were not measured in the SP buildings (one test in a SP building was conducted to check that nicotine was not detectable), or in the non-smoking areas of the RSV buildings. Nicotine concentrations differed among the areas with spatially restricted policies, whether the RSV policy was included in the statistical analysis ($F_{2,12}=8.22$, $P=0.0056$) or excluded from it ($F_{1,9}=12.90$, $P=0.0058$). The areas applying the RF policy had the highest concentration of nicotine (Table 3). There was a significant interaction between smoking area designation and policy ($F_{1,65}=14.88$, $P=0.0003$). The nicotine concentration was significantly greater in the smoking areas than in the office areas for the RF and RNT policies, but the concentration in the smoking areas of the RF policy was more than four times that of the RNT policy, whereas the nicotine concentrations in the office areas were very low for both policies.

Partial correlations were calculated using the data from the spatially restricted policies to assess how tightly coupled nicotine concentrations were with other ETS pollutants. Only u.v.PM was positively correlated with nicotine ($r=0.32$, $P=0.0057$). Using the data relating to all policies $RSP_{3.5}$ correlated with u.v.PM ($r=0.36$, $P=0.0001$), CO ($r=0.23$, $P=0.005$) and CO_2 ($r=0.29$, $P=0.0003$). CO and CO_2 were positively correlated ($r=0.26$, $P=0.0011$). RH was positively correlated with formaldehyde ($r=0.23$, $P=0.0050$) and with CO_2 ($r=0.33$, $P=0.0001$), and negatively correlated with temperature ($r=-0.17$, $P=0.0341$).

There were no significant effects of smoking policy on CO_2, temperature, relative humidity or illumination. Analysis of only the spatially restricted smoking policies showed higher concentrations of CO_2 ($F_{1,88}=5.89$, $P=0.0173$) and lower levels of illumination ($F_{1,88}=4.02$, $P=0.0481$) in the smoking areas (Table 4).

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Effects of prohibiting smoking vs allowing smoking on indoor environment conditions

To test the effects of prohibiting smoking vs allowing smoking on environmental conditions, data for the SP and RWS policies were compared (in SP buildings there was no smoking and in RWS buildings smoking could occur at any desk). No significant differences between the SP and RWS policies were found in concentrations of CO, CO_2, metered RSP or formaldehyde, or in levels of illumination, or temperature or RH, but u.v.PM levels were higher for buildings where the RWS policy was operated ($F_{1,10} = 38.75$, $P = 0.0001$: Table 5).

DISCUSSION

Few differences in indoor air quality were found among the five smoking policies studied, and the pollutant concentrations which were found are comparable to those of other studies of the effects of smoking policy on indoor air quality (STERLING *et al.*, 1987; OLDAKER *et al.*, 1992). Concentrations of ETS 'tar', as measured by u.v.PM, and respirable particulates (3.5 μm) were lower for the smoking prohibited policy than for the restrictive smoking policies. However, the amount of particulate material was small relative to current occupational exposure standards for respirable particulates. Nicotine concentrations also varied among the restrictive smoking policies, and this was correlated with u.v.PM, which measures the 'tar' particulates of ETS. Formaldehyde concentrations generally were very low in the study buildings, and were unaffected by differences in smoking policy. Because indoor air quality was assessed using integrated air sampling performed in general office areas, these results indicate the average concentrations which a non-smoker could be exposed to in the office. The air sampling techniques which were used did not measure transient changes in pollutant concentrations around point sources, such as the immediate change in pollutant levels which might be expected in the micro-environment of an active smoker. The results show that for ETS associated pollutants, ambient indoor air quality in non-smoking office areas in air-conditioned office buildings which spatially restrict smoking can be comparable to that in buildings which prohibit smoking.

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MEASUREMENTS OF THE EFFECTIVENESS OF DUST EXTRACTION SYSTEMS OF HAND SANDERS USED ON WOOD*

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Abstract—An investigation has been made of the production of dust by hand sanders when used without dust extraction, with integral dust extraction and with external dust extraction. The apparatus used for the measurement was designed to carry out sanding in a repeatable manner, simulating normal working practice. The geometry of the wood sanded affects the dust concentration critically, whereas the effects of the sandpaper grade and of the filtration efficiency of the collection system are small. Belt sanders produce high dust levels in all situations, but they are very efficient in removing wood, which means that they need to be used for only a short period. The integral systems of orbital sanders give good dust control on flat wood but not on edges. The high air extraction rate of external systems results in much better dust control during the sanding of edges, though the performance of the two extraction systems on flat wood is broadly similar. The size distribution of the dust clouds produced by the sanding is relatively constant, but that produced by rotary sanders tends to be coarser than that produced by orbital sanders, consistent with the smoother finish given by the latter. The electric charge on the dust produced by sanding is high.

INTRODUCTION

PROLONGED exposure to high levels of wood dust can cause health problems. FRIAR *et al.* (1980) found that the incidence of nasal cancer was 1000 or more times greater amongst woodworkers than amongst the general male population. BOLM-AUDORFF *et al.* (1989) carried out a general study of nasal and nasopharyngeal cancer, and found that 5 years' exposure to wood dust increased the risk of such diseases by a factor of about 8. NORPOTH (1989) found an association between long-term exposure to certain hardwoods (beech, oak and chestnut) and cancer in organs remote from the respiratory system, such as the colon and the haemopoietic system. PISANIELLO (1989) listed the conditions that the dust can cause, such as dermatitis, sore or itchy eyes, allergic and non-allergic respiratory effects, sino-nasal effects such as blocked or bleeding nose, and cancer in the nose and elsewhere.

Correlations between the incidence of symptoms and levels of exposure are quite poor, probably because many of the effects depend on the sufferer's history. Exposure to hardwood dust appears to be associated with a greater incidence of many of the symptoms than does exposure to softwood dust. Nevertheless, the softwood, western red cedar, is known to be highly allergenic; and other soft woods cause dryness in the nose, and general eye, nose and throat irritation. Workers exposed to wood dust also complain of prolonged colds and frequent headaches.

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SECTION 4

ETS AND LUNG CANCER

BIOLOGICAL MEASUREMENT AND DOSIMETRY

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SCOTH REVIEW - VOLUME 3

SECTION 4

BIOLOGICAL MEASUREMENT AND DOSIMETRY

Reasor (1992) states that biological markers have been identified which can serve as quantitative surrogates for ETS exposure. Nicotine and its metabolite cotinine have been used for this purpose by many workers. However, nicotine is pertinent only to the gas phase of ETS and does not correlate with particulate retention. The usefulness of cotinine as a biomarker for ETS is limited in the first instance by its derivation from nicotine exposure. Furthermore, cotinine in body fluids is not even a reliable indicator of nicotine uptake because of variations in metabolism among individual subjects.

Other potential biomarkers, such as DNA adducts and chromosome aberrations have in several studies been reported not to be increased significantly in ETS - exposed non-smokers compared to non-exposed non-smokers (nor in ETS-surrogate animal inhalation studies; see Section 5). Furthermore, the relevance, if any, of such changes to lung cancer is not fully understood (Scherer, 1993).

The uptake of chemicals present in ETS is not well understood, and the US EPA commented that it was too early for studies on this subject to be useful. It is noteworthy, however, that according to Holcomb (1993), "the estimated dose of ETS one can be expected to receive does not support the health risk claims being made by the US EPA and others."

SECTION 4

BIOLOGICAL MEASUREMENT AND DOSIMETRY

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Toxicology of Combustion Products
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Toxicology of Environmental Tobacco Smoke

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Abstract

Environmental tobacco smoke (ETS) is a complex and dynamic mixture of particles and gases which has been poorly characterized. Most experimental research has involved study of sidestream smoke rather than ETS, therefore, such results are difficult to interpret relative to human exposure.

Biological markers, including cotinine in biological fluids and DNA and protein adducts, have been utilized to assess exposure to ETS; however, none has been identified that can serve as a quantitative surrogate for ETS. As a result of the paucity of information regarding ambient ETS characterization and exposure assessment, it has been difficult to evaluate the possible toxicological effects of ETS on humans. Development of relevant studies involving animal and "in vitro" exposures may provide important information on the toxicology of ETS.

Introduction

ETS is a complex aerosol of gases and particles originating from the smoke exhaled by the active smoker and all other material released by the burning tobacco product. The possible health effects of ETS exposure have been the subject of much public and scientific interest in recent years.

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Unfortunately, a meaningful evaluation of the potential toxicological impact of ETS exposure has been hampered by incomplete information on the composition and dynamics of ETS as well as inadequate exposure assessment.

The present manuscript focuses on three areas in which additional information is needed to evaluate the toxicology of ETS: (1) the chemical composition and dynamics of ETS, (2) ETS exposure assessment, and (3) animal and "in vitro" studies in assessing the potential toxicity of ETS.

Chemical composition and dynamics of ETS

The following discussion will be confined to cigarettes only. ETS originates from both the smoke exhaled by the smoker (exhaled mainstream smoke) and all of the smoke that is emitted by a burning cigarette that is not mainstream smoke (sidestream smoke), 95% of which is emitted from the tip of the cigarette between puffs (1). Comprehensive discussions of the physical and chemical aspects of sidestream smoke generation have been presented by Baker and Proctor (1) and Guerin (2).

Most research efforts at characterizing ETS have involved the use of sidestream smoke generated from smoking machines under standardized conditions (3-5). Over 100 chemicals have been identified in sidestream smoke (6). Values for each vary depending on the conditions used r generation and collection. This is further illustrated by the wide range of values reported for chemicals measured in sidestream smoke (6). In an indoor environment sidestream smoke undergoes significant chemical and physical changes as it contributes to ETS (7). These changes are termed "aging" and contribute significantly to the complex and dynamic nature of ETS. Aging is influenced by a number of factors including the type of tobacco smoked, chemical reactions, dilution, evaporation, ventilation, temperature, humidity, lighting, and deposition onto surfaces. As a result, the properties will differ depending on conditions at any given time, thus making it impossible to provide a definitive chemical and physical description of ETS. For the various reasons described, it is not appropriate to directly extrapolate information on sidestream smoke to the qualitative or quantitative characterization of ETS. These problems not withstanding, considerable information exists on sidestream smoke as a surrogate for ETS, however, such data must be evaluated in the proper context. Several observations illustrate the variable and dynamic features of sidestream smoke-derived ETS (1, 6). It has been noted that nicotine, which is found predominantly in the particulate phase of mainstream smoke, is almost exclusively located in the gas phase of ETS. Materials in ETS decay at different rates

in the following order from highest to lowest rates: nicotine > particles > total hydrocarbons = NO > CO_2 = CO > NO_2. Particles may become smaller through evaporation, they may increase in size through coagulation, and they may be lost by deposition on surfaces. Considering the more extreme nature of an ambient indoor environment, it is likely these changes would be even more pronounced and unpredictable.

ETS exposure assessment

Monitoring exposure with biological markers

While monitoring the indoor environment for ETS may provide insight into the potential for human exposure, it is difficult to draw conclusions from such data on the internal dose received. Accordingly, biological markers have been used as surrogates to qualitatively and quantitatively assess internal exposure to ETS. A major problem with such an exposure assessment is that it has been possible to monitor exposure to only a few of the multitude of chemicals in ETS, thus only a limited amount of toxicologically-relevant information has been obtained.

Nicotine, and its metabolite cotinine, in biological fluids have been widely used as qualitative and quantitative markers for ETS exposure (8). In general, nicotine is a measure of very recent exposure, while cotinine has a longer half life. Of the two markers, cotinine in urine or saliva appears to provide the best correlation with self-reported exposure (9). Nevertheless, there are significant limitations in the use of cotinine as a quantitative measure of ETS exposure (10). In nearly all of the studies reported, single samples of body fluid are collected. Such a protocol precludes assessment of chronic ETS exposure. Cotinine is a measure of exposure to gas-phase nicotine, and consequently, provides no information on other gas-phase constituents nor on particulate-phase chemicals. Additionally, cotinine is only one of several metabolites of nicotine and may not be the most abundant nor most consistent to measure.

Exposure to low levels of nicotine may occur independently of ETS. Recent evidence exists that nicotine is present in a number of vegetables in our diet (11), therefore, the low-level cotinine values observed in nonsmokers may not exclusively be a reflection of ETS exposure. Nicotine has been detected in house dust in the homes of smokers and nonsmokers (12). A potential source of nicotine-derived markers is from nicotine-containing chewing gum used to help reduce smoking. Tobacco-specific nitrosamines have been detected in the saliva of persons chewing "Nicorette" gum (13). It is certainly possible that

low levels of cotinine in the body fluids of nonsmokers may arise from sources other than ETS.

DNA and protein adducts have been utilized as biological markers to assess internal exposure to ETS (14). The two adducts that have been monitored are 4-aminobiphenyl hemoglobin in the blood and benzo(a)pyrene diol epoxide-1-DNA in peripheral blood cells. In addition to being present at low levels, neither adduct reflects exposure to a tobacco-specific constituent, thereby making it difficult to ascribe their presence to ETS exposure.

Toxicology of ETS

Toxicological aspects concerning ETS exposure in humans are an area of ongoing debate and controversy. A number of reports have appeared alleging that chronic exposure to ETS results in adverse health effects in children and adults (15, 16). A body of literature exists which has provided strong scientific reasoning in dispute of that conclusion (17-19). The principal reason for this controversy involves the nature of the human studies which have been almost exclusively by epidemiological procedures. Epidemiology is notoriously weak at establishing causal relations at the low relative risks reported in studies involving ETS exposure.

It is unlikely that this controversy will be resolved by dependence on further epidemiological studies; alternative approaches will have to be utilized including studies using animals and "in vitro" systems. In contrast to the abundance of epidemiological studies concerning ETS exposure, virtually no relevant information exists on the effects of ETS in animals and "in vitro" systems. In studies using animals, the protocols generally have involved exposure to only sidestream smoke and at levels that are unrealistically high compared to ambient exposure to ETS (Table 1). As a result, it is difficult to interpret the results of these studies in the context of human exposure. Increased emphasis in ETS research should be placed on developing and utilizing whole animal and "in vitro" exposure systems and protocols utilizing conditions simulating ambient exposures.

It has been suggested that ETS is just a dilute form of the mainstream smoke inhaled by the active smoker, and therefore, in attempting to understand the possible effects of ETS, it is valid to extrapolate from what is known about active smoking. There is no evidence to support such an assertion. While mainstream smoke is highly concentrated, and its properties are rather well characterized, ETS is exceedingly more dilute and far more dynamic. Thus, it seems apparent that comparison of ETS exposure to active smoking in a toxicological context is of little value (19).

Table 1.
Experimental studies of animal exposures to sidestream smoke.

Species	Level of Exposure: Biomarker	Level of Exposure: Air	Citation
Mice	5.95% & 11.25% COHb[1]	—	(20)
Rats Hamsters	3.3% COHb 3.1% COHb	25 ppm CO[2]	(21)
Hamsters	—	2,000 ppm CO	(22)
Mice Rats Guinea Pigs	17.9% COHb 19.1% COHb 40.2% COHb	—	(23)
Rats	4.6-20.0% COHb	—	(24)
Guinea Pigs	39.4% COHb	—	(25)
Hamsters	5-15% COHb (approx.)	—	(26)

[1] Carboxyhemoglobin; [2] Carbon monoxide.

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Commentary

TITRATING EXPOSURE TO TOBACCO SMOKE USING COTININE—A MINEFIELD OF MISUNDERSTANDINGS

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DEFINITION OF THE PROBLEM

Whilst epidemiologists disagree about many things, there seems to be universal approval for the notion that objective measurement of tobacco smoke exposure is considerably more desirable than more self-reporting of smoking habits. As with all dogmata, caveats abound, one of the most practical of which is that without sacrificing reliability, the field test should be both simple and cheap. For reliability to be conserved, the ideal test would distinguish between true non-smokers, non-smokers exposed to environmental tobacco smoke (passive smokers) and smokers; maybe even between different smoking habits and consumption rates. Thus the test would have absolute sensitivity and specificity. It has become quite clear over the last two decades that, as various candidate tests have been introduced, the panacea would not be found. Pretenders to the crown, such as expired carbon monoxide, percent carboxyhemoglobin and thiocyanate concentration in various body fluids, have failed to accede because of the ambiguities of incomplete sensitivity and specificity. Contemporary laboratory technology has answered the clarion call by developing such methodology as capillary gas chromatography–mass spectrometry of 4-aminobiphenyl-hemoglobin adducts [1], gas chromotography–thermal energy analyzer detection of urinary *N*-nitrosamino acids [2] or ^{32}P-postlabeling tests for smoking-related DNA adducts [3]. Whilst such analyses embody contemporary methodology coupled with great precision, by their very nature, they fall outside the scope of typical clinical epidemiologic survey which simply wishes to relate a measure of tobacco smoke exposure to health issues. It seems that rather by default, salivary and urinary cotinine determinations have evolved as the current touchstones of tobacco exposure. Apart from its simple determination, and the availability of a rapid result at low cost, there is the added appeal that cotinine, as a relatively long-lived metabolite of nicotine, can be detected in the aforesaid fluids long after the "culprit" nicotine has dwindled to meagre levels.

Because the greatest body of experience to date with cotinine surrounds epidemiologic enthusiasts, often with scanty training in drug metabolism and pharmacokinetics, the two disciplines which underpin the basis of the use of cotinine in this context, I believe that a critical review of the premises and procedures is required in an attempt to examine the misconceptions which can lead to overinterpretation in what is otherwise an exciting new field of biochemical epidemiology.

My own dissatisfaction with indiscriminate use of cotinine as a dosimeter of tobacco smoke arises from the trade-off of knowledge for convenience. Nicotine is a pyridine alkaloid, one of at least 10 identified in cigarette smoke [4] and both the qualitative and quantitative distribution of this class of alkaloids amongst the flora, their metabolic interconversions in man, the issue of the concentration of cotinine in the saliva at the expense of plasma, together with

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the extent of intersubject variability in human disposition of nicotine and its metabolites is both nebulous and poorly-understood. The complex of dynamic interactions which leads to a certain salivary or urinary concentration of cotinine at one point in time following exposure to a defined amount of airborne nicotine needs to be dissected. The purpose of this commentary is to demonstrate by such a dissection that single point cotinine concentrations can give no more than a clue to a past exposure to pyridine alkaloids of unknown amount, at an unspecified time, by an unknown route of entry and from unknown origins.

SOURCES OF NICOTINE AND COTININE

One of the principal premises of the practice of cotinine biomonitoring is that nicotine, and hence cotinine, is a tobacco-specific alkaloid. Cotinine is variously described as "a particularly specific and sensitive marker of exposure to tobacco smoke" [5], "a useful and reliable indicator of nicotine intake" [6] and "a reliable indicator of tobacco smoke exposure" [7]. These statements deserve further comment in the light of the recent finding of nicotine both in *Solanaceae* plants which are consumed as vegetables in our diet and in instant tea preparations [8]. Whilst nicotine, with the exception of carbon monoxide, is the most abundant single chemical in tobacco smoke, with an estimated yield of 1.0–2.3 mg per cigarette [9], with a mean nicotine intake per cigarette calculated as 0.75–1.25 mg per cigarette [10], it can no longer be considered as tobacco-specific. Significant nicotine concentrations (mg/kg dry weight) have been found in tomatoes (1.5–3.2), potato peel (9.5–16.1), eggplants (1.9–3.0), green peppers (1.3–3.9), green tea (1.8–2.4) and two brands of instant tea (12.2–28.0). It is possible to calculate from these data that a person who consumed 10 cups of tea in a day, ate food comprising 1 lb of tomatoes, aubergines or peppers and 1 lb of potatoes with their skins, might ingest nicotine equivalent to 1–2 cigarettes. The confounding effect, particularly in vegans and vegetarians, upon cotinine biomonitoring of environmental tobacco smoke exposure, might be considerable. It is hardly surprising that imported foodstuffs might contain high levels of nicotine, when nicotine is still widely used in the developing world as a cheap and effective insecticide. The data so far available though, suggest that nicotine is elaborated by the *Solanaceae* such as tomatoes, since it is found not only in the fruit, but also the leaf, stem and root [8].

Whilst tobacco smoke contains high concentrations of nicotine, it also contains cotinine (9–57 μg per cigarette) and nornicotine (27–88 μg per cigarette) [4], both of which are mammalian metabolites of nicotine. Because nornicotine can be methylated in the lung to give nicotine [11], cotinine biomonitoring will reveal not only exposure to tobacco smoke and dietary nicotine, but also to cotinine itself and to nornicotine. Whether or not these latter two related alkaloids occur in vegetables or as degradation products of environmental nicotine insecticides is not known.

HUMAN METABOLISM OF NICOTINE AND COTININE AND ITS INTERSUBJECT VARIABILITY

It is generally assumed that cotinine arises from the metabolic oxidation of nicotine in human tissues by cytochrome *P*-450, although which discrete one of the myriad of *P*-450 isozymes effects this reaction is unclear. What is certain is that at any given time the concentration of cotinine in the plasma will depend upon not only the dose of nicotine ingested and inhaled, but is rate of conversion to cotinine, the rates of competing metabolic transformations to nornicotine and nicotine *N*-oxides, the rate of onward metabolism of cotinine to its own metabolites, together with both the rates of excretion of nicotine and cotinine in the urine and any sequestration of the two compounds within other body compartments which occurs. All these complex interactions are then candidates for intersubject variability arising from physiological, environmental, pathological and genetic differences which exist between all of us. What is so surprising, considering the perceived importance of nicotine, is that the balance of its metabolic transformations in man is not known: only a fraction of its metabolites have been identified.

For many years cotinine was considered to be the principal metabolite of nicotine and indeed many authors refer to it as such [10, 12–14]. However, recent studies point to *trans*-3'-hydroxycotinine as the major metabolite of nicotine [15]. Parviainen and Barlow [16] have criticized this interpretation of the chromatographic data and themselves refer to "metabolite 5" which behaves similarly to the

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trans-3′-hydroxycotinine of Neurath and Pein [15]. Further studies by these authors [17, 18] seem to establish the pre-eminence of *trans*-3′-hydroxycotinine amongst the known nicotine metabolites. Our own studies have shown that the *N*-oxides of nicotine are more abundant in the urine than cotinine after nicotine ingestion [19]. In studies where sufficient raw data were given to assess the extent of variability in cotinine levels under constant smoking conditions, the amount of intersubject variation observed is large. For example, the data cited by Neurath and Pein [15] for 9 subjects, who each smoked 19 cigarettes (each 1.35 mg nicotine) per day for 6 days, show that the plasma nicotine concentrations varied from 7–44 ng/ml, the cotinine from 41–344 ng/ml and the *trans*-3′-hydroxycotinine from 24–160 ng/ml, giving 6-, 8- and 7-fold variations respectively, with the greatest for cotinine. Similarly in the urinary studies of Cholerton *et al.* [19], cotinine excretion displayed the highest coefficient of variation of any metabolite. Cytochrome *P*-450-mediated metabolic oxidations are frequently subject to genetic polymorphism [20, 21] and as yet undefined genetic polymorphism may underlie this observed variation. What can be said is that plasma and urinary concentrations of cotinine do not reflect the dose of nicotine entering the body.

DISPOSITION OF COTININE IN SALIVA

In spite of the fact that a wide variety of drugs is subjected to therapeutic monitoring, for very few is this routinely done so in saliva instead of in plasma. It is only really for certain anticonvulsant drugs that the "non-invasive" salivary protocol has become a reality. It is noteworthy that Danhof and Breimer [22], in their review of therapeutic drug monitoring in saliva, conclude "In single dose studies, many discrepancies in the saliva/plasma ratio have been described, especially during periods of drug absorption. It is probably not possible to conduct reliable pharmacokinetic and biopharmaceutical single dose studies based upon saliva data alone". The subsequent decade has witnessed a gradual abandonment of the use of saliva in therapeutic drug monitoring. Nevertheless, salivary cotinine continues to be popular with the epidemiologists [23–25].

Two characteristics of a drug determine its penetration into saliva, its ionization constant expressed as a pK_a value and the fraction unbound to plasma proteins. In the case of nicotine, both nitrogen atoms are ionizable with pK_as of 8.02 and 3.12 [26], meaning that nicotine is both mildly and weakly basic. Cotinine however has lost its mildly basic pyrrolidine nitrogen to become a lactam, leaving only the weakly basic pyridine nitrogen with a pK_a of 4.37 [27].

Unlike nicotine, which is too basic, cotinine, with a pK_a of less than 5.5, is now able to freely enter saliva [28]. However, there is some contention that cotinine enters too freely, and is somehow concentrated by the gland, giving artificially high estimates of the plasma concentration [7], although this has been the subject of a strong debate [29, 30]. It is not unusual for drugs to be concentrated in saliva and this is one of the hallmarks of lithium which is actively transported into saliva to reach concentrations 2.2–3.3 times higher than plasma [22]. Active transport has also been proposed for both phenytoin and penicillin [22]. Accordingly either single spot concentrations or pharmacokinetic profiles of cotinine in saliva may over-represent the true disposition of the compound in plasma, thus clouding interpretation of such data.

ANALYTICAL DETERMINATION OF COTININE

A constellation of individual methodologies has been published. Many determine nicotine concentrations simultaneously. Many of the most sensitive, specific and reliable assays require the participation of a mass spectrometer [12, 31, 32] which is not available to most researchers. Gas chromatography using nitrogen-sensitive detection has also been employed [14, 33]. The ubiquitous HPLC has been applied to the problem [13, 16, 34, 35]. Probably the biggest advance has been the appearance of an enzyme-linked immunosorbant assay (ELISA) using a monoclonal antibody which recognizes cotinine [36]. Although only minimal cross-reactivity with nicotine and nicotine metabolites occurs, until the discovery by Neurath and Pein [15] that *trans*-3′-hydroxycotinine was the principal metabolite of nicotine hitherto, no assessment of cross-reactivity between the commonly-used anticotinine antibodies and *trans*-3′-hydroxycotinine was performed. Indeed this major metabolite cross-reacts by about 30% with the polyclonal rabbit anticotinine antiserum commonly used to determine cotinine levels by ELISA [37].

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USES AND ABUSES OF COTININE

In situations where the cotinine concentrations are massively elevated, such as in the urine, blood and saliva of active smokers, cotinine determinations should in principle serve a useful purpose in verifying self-reported smokers and non-smokers. The experiences of Jarvis *et al.* [24] are salutary in this regard. These investigators studied 215 outpatients attending cardiology and peripheral vascular clinics where a reasonably high proportion of cigarette smokers were anticipated. Accurate self-reporting of smoking status was encouraged by guaranteeing confidentiality and anonymity of responses. Eleven different biomarkers of smoking were determined on each subject including expired carbon monoxide and %COHb; plasma, saliva and urine thiocyanate, nicotine and cotinine. Plasma cotinine gave the best discrimination between self-reported smokers and non-smokers, but nevertheless 21 self-reported non-smokers had plasma cotinine levels similar to the smokers (cut-off 13.7 ng/ml cotinine). These 21 anomalous individuals were labeled "Deceivers" by these authors, even though 5 of them did not have raised thiocyanate levels and 3 had expired CO levels below the smoking cut-off of 5.6 ppm. Presumably the anonymity also protects the authors from litigation. What is most surprising is that no comment is made by the authors about the 9 self-reported smokers who had plasma cotinines *below* the cut-off of 13.7 ng/ml. In spite of its better performance than other biomarkers tested, plasma cotinine could still be seriously wrong on an individual case basis in this study. Using pejorative terminology such as "deceiver" to label a patient as a liar is in my view not only highly invasive of that patient's human rights but also unscientific. It may very well be that, by trading knowledge for convenience, an unusually high dietary intake of nicotine and cotinine has been overlooked, or a pharmacogenetic variant in nicotine/cotinine metabolism has stealthily been at work, or for that particular sample the assay did not perform adequately. This may be particularly important when issues of minor nicotine intake, such as environmental tobacco smoke, are being considered, where the signal-to-noise for cotinine values might be seriously confounded and compromised by diet and pharmacogenetic variation. Again the literature contains such possible examples: 330 non-smoking adolescent schoolgirls in south London were studied by Jarvis *et al.* [38]. They were partly categorized as non-smokers by having salivary cotinine concentrations of less than 14.7 ng/ml [24]. The authors claim that the high correlation ($r = 0.75$; $p < 0.0001$) between non-smoking girls' salivary cotinine concentrations on two occasions 1 year apart was due to a constant home environment where either or both parents smoked indoors. Thus, the pattern of salivary cotinine due to the breathing of one or both parents' smoke was maintained with high reproducibility over 12 months. At least this is the interpretation put on the findings by the authors and they may be right, but they have ignored the possibility that an individual's salivary cotinine level is fixed by a combination of environmental, physiological and genetic factors and not merely by one exposure factor. From my perspective as a pharmacogeneticist a great deal of work is yet required before such weight can be attributed to salivary cotinine concentrations as is often witnessed. The full metabolic picture of nicotine is not known, the extent to which single genes can determine individual patterns is unknown, the complete dietary spectrum of nicotine and related pyridine alkaloids is unavailable at present, and the ability of salivary glands to concentrate cotinine is still under debate. I believe that the time is right to trade some expediency for proper investigation of the problem.

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Correlating exposure to environmental tobacco smoke exposure with increased incidence of lung cancer in non smokers : is cotinine a valid marker ?

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ABSTRACT

Environmental Tobacco Smoke (ETS) is a complex mixture of Exhaled Mainstream (EMS) and Sidestream Smoke (SS) composed of gases and Respirable Suspended Particles (RSP). It is both a highly diluted and an aged mixture the composition of which is difficult to assess. Based mainly on syllogisms it has been hypothesized that exposure to low levels of ETS increases the risk of lung cancer in nonsmokers. There is no question that nonsmokers living in the presence of smokers are exposed to tobacco smoke constituents. But, to conclude on the significance of such an exposure, in term of increased incidence of lung cancer in non smokers, can only rely on a quantitative biomonitoring of exposure to agents proven to play a causative role in lung carcinogenesis. Nether nicotine nor its metabolites are likely to be precise markers of exposure to such chemicals. Molecular epidemiology offers a promising, but still to be validated, tool to meet such a challenge by measuring proteins and/or DNA adducts in serum or urine samples.

Key words: tobacco smoke, cotinine, lung cancer, cancer risks.

INTRODUCTION

ENVIRONMENTAL TOBACCO SMOKE (ETS) is a complex mixture of exhaled mainstream smoke (EMS) and sidestream smoke (SS) composed of gases and respirable suspended particles (RSP) [see Table 1 (1-3) for definitions]. It has been hypothesized (4,5) that exposure to low levels of ETS increases the risk of lung cancer in non smokers. Two syllogisms support this hypothesis: [1] a) if ETS is chemically analogous to mainstream cigarette smoke (MS) ; and b). MS is known to cause lung cancer in active smokers; then c). ETS causes lung cancer in passive smokers; [2)] a): if ETS contains lung carcinogens, and b): it is generally assumed that no dose threshold exists for carcinogens: then c). ETS is a lung carcinogen. There is no question that non smokers living in the presence of smokers are exposed to tobacco smoke constituents. Self-reported exposure to ETS as well as the use of biomarker like nicotine or cotinine are certainly valid means to help epidemiologists register the extent of such exposure. However, its significance in terms of increased incidence of lung cancer in non smokers cannot simply be derived from syllogisms. For a molecular toxicologist, such a conclusion can only rely on a quantitative biomonitoring of exposure to agents proven to play a causative role in lung carcinogenesis.

Indeed, since the exposure level to ETS is likely to be low compared to active smoking (3,6), since many confounding factors (like radon exposure, misclassification of light smokers, former smoking habits, and dietary factors) are likely to exist, one has to recognize that the classic epidemiological methods alone may not be sufficiently sensitive for scientifically establishing that ETS exposure plays a significant role in the incidence of lung cancer in ETS-exposed non smokers (3). Such a conclusion needs to be supported by dosimetry which not only confirms personal records of ETS exposure in quantitative measures of a reliable parameter, but also demonstrates that such an exposure has indeed led to a significant intake of carcinogenic components. Various biomarkers of exposure to cigarette smoke [see Table 2 (3; 7-10)] have already been proposed with the aim of developing a quantitative approach to the epidemiology of diseases in non smokers, particularly lung cancer. These include measures of plasma, salivary and/or urinary concentrations of either (a) a major, specific tobacco smoke molecule and/or its main metabolites or (b) the end-products of the metabolic activation of carcinogenic components in tobacco smoke.

The aims of the present paper are :

1) to review the data on the quantitative biomonitoring of exposure to ETS based on nicotine and/or cotinine measurements in physiological fluids.

Definitions

Environmental tobacco smoke or ETS
A mixture of diluted and aged gases and solid suspended particles resulting from a combination of sidestream smoke (SS) (80-90%) and exhaled mainstream smoke (EMS) (10-20%);

Mainstream smoke or MS:
The cigarette smoke drawn through the tobacco into smoker's mouth;

Exhaled mainstream smoke or EMS:
The fraction of MS not retained by the smoker and exhaled in the air (EMS is not identifical to MS because it has been substantially depleted in vapor phase constituents and because the particulate matter is likely to have increased its water content);

Sidestream smoke or SS:
The smoke emitted by burning tobacco between puffs and/or the aerosol emitted in the surrounding air from a smoldering tobacco product between puff drawing and/or all of the tobacco smoke issued apart from the MS which is delivered to the smoker.

Respirable suspended particles or RSP
The solid phase of the aerosol.

Table 1: Definitions (1, 2, 3)

2) to discuss the relevance of these biomarkers in assessing any increase in lung cancer incidence associated with exposure to ETS.

3) to propose new approaches likely to be more scientifically relevant to evaluate exposure to ETS for risk [illegible]

BIOMONITORING ETS EXPOSURE: A REVIEW OF THE DATA

The [illegible] assessment of exposure to ETS should [illegible] from measurements of (a) relevant biomarkers made on easily accessible physiological fluids by using a simple, readily available analytical method. Biomarkers are "a measure of dose or uptake and hence an indicator that an exposure has taken place" (5).

An ideal biomarker of exposure to ETS (5, 10) must:

a) be chemically specific to ETS;
b) have a long half-life in the body;
c) be present in sufficient quantity in tobacco smoke so that it can be measured even at low levels of ETS exposure;
d) be characterized by similar emission rates for a variety of tobacco products;
e) be in a fairly constant ratio to different combustion products of tobacco smoke constituents accross different brands of cigarettes and for a range of environmental conditions encountered;
f) be either the agent which is associated with the suspected effect(s) or strongly associated with the agent of interest.

Of the biomarkers [Table II (3, 7-10)] which have, up to now, been developed to a significant stage to monitor tobacco smoke exposure by active smokers, only nicotine and its metabolite cotinine have been used repeatedly to monitor exposure to ETS. Indeed, they have been proposed as the most specific and the most sensitive markers presently available (11). But, as underlined in the US-EPA document (5): "although these biomarkers (may) demonstrate that an exposure has taken place, they may not be directly related to potential for development of the adverse effect under study".

Biomarker	Comments*
carbon monoxide carboxyhemoglobin thiocyanate	not tobacco specific not sufficiently sensitive
urinary thioethers urinary mutagens	not tobacco specific contradictory results
solanesol	not enough date yet available to validate
N-nitrosoproline N-nitrosothioproline aromatic amines	lack of data and sensitivity as well as reliability of analytical procedures
protein and NDA adducts	promising technique, but still to be validated (see this paper)
nicotine, cotinine, and 3-hydroxycotinine	see this paper

Table II. Major biomarkers of tobacco smoke exposure: state of the art.
*These comments have been taken directly from the references and they express the current scientific concensus.

Data (Table III and IV; 7, 12-23) show that non smokers [illegible] ETS [illegible]

[illegible] urinary excretion are practically useless because they are given as ng/[illegible] (24-27), a parameter [illegible] of any physiological significance. Only data [illegible] as ng of cotinine/mg of creatinine or ng of [illegible]

biomarker ETS exposure biological fluid	nicotine no	 yes	cotinine no	 yes	references
saliva ng/ml			2.0 (0.0-1.9)	3.5-4.2 (0.0-16)-(0.0-[illegible])	12
			1.8 (0.3-1.5)	1.8 (0.0-1.5)	13
	1.9	43.6	1.5	5.0	14
			0.7	2.2-2.6	[illegible]
			0.44 (0.7) (0.0-0.9)	[illegible] 0.4-1.9-1.5-4.4	15
	0.0	12.7 (0-166)	0.0 (0.0-3.0)	9.0 (0.0-25)	16
plasma ng/ml	0.76	2.5	1.1	[illegible]	14
			1.1 (0.5)[a]	4.8 (7.7)[a]	17-18
			0-0.4 (1.0)	1.1 (0.3)-4.9 (0.9)	19
	1.0	0.75-0.9	0.8	1.8	15

Table III. Nicotine and cotinine concentration in saliva and plasma of non-smokers: review of published data.
- ETS exposure (yes) is either in experimental (13, 14, 17-19) or normal life (self reporting) (7, 12, 15, 16) conditions.
- a=peak values estimated from graphs.
- values in ( ) are range of values given as medians.
- figures in italics are standard errors.

biomarker ETS exposure biological fluid	nicotine no	 yes	cotinine no	 yes	references
Urine[b] ng/mg creatinine			2.7-3.1	6 (0.0-100)	20
	0.0 (0.0-59)	53 (0.0-370)	4.0 (0.0-125)	351 (41-1885)	16
			510 (90) 220 (70)	790 (100) 720 (100)	21
				50	22
				60 (24-117)	23

Table IV. Nicotine and cotinine concentration in urines of non-smokers: review of published data.
- Ets exposure (yes) is either in experimental (13, 14, 17-19) or normal life (self reporting) (7, 12, 15, 16) conditions.
- b=values which have been reported as ng cotinine/ml of urine or µg cotinine/24h in (6, 7, 14, 19, 24-27) have not been included.
- values in ( ) are range of values given as medians.
- figures in italics are standard errors.

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nine/24h are meaningful. The general conclusions of these studies are that:

a) the experimental data are not always comparable in the different studies, in particular with regard to uri-

because of its longer half-life, cotinine is likely to be a more suitable marker than nicotine.

salivary and urinary concentrations of cotinine correlate with serum concentrations.

d) passive smokers have, in their physiological fluids, concentrations of cotinine equivalent to few ng/ml or ng/mg creatinine as compared to hundreds or thousands of ng/ml or ng/mg creatinine in active smokers;

e) such parameters might be valid for estimating acute exposure to relatively high levels of ETS;

f) at low levels of exposure to ETS, non-smokers have concentrations of nicotine and/or cotinine in their biological fluids, which are not very different from those measured in non-smokers not exposed to any identified ETS [(Table III and IV (7, 12-27)]. As early as 1984, Jarvis *et al.* (7) reported that "the majority of non-smokers in UK may have measurable amounts of tobacco-specific chemicals in body fluids". Proctor *et al.* (13) have also expressed their "doubts about the validity of salivary cotinine at low levels". Moreover, in a large nationwide survey conducted in the US under the auspices of the CDC's National Center for Environmental Health and the National Center for Health Statistics (28), 100% of the 800 persons so far analysed were reported to have measurable levels (0.03 to 650 ng/ml) of cotinine in their serum. There is no reason to believe that all these people either smoke or are exposed to ETS. If confirmed, such an observation would have important practical consequences. Indeed, when correlating urinary cotinine as a biomarker of ETS exposure with increased risk of lung cancer, one would have to refer to increased urinary cotinine concentrations (meaning the difference between ETS exposed and non-exposed individuals) but not to absolute values.

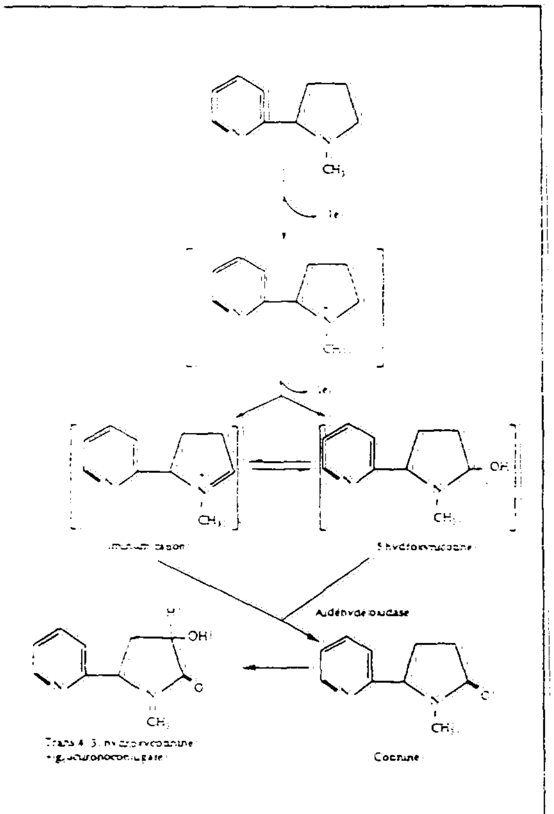

Fig. 1 Major metabolic pathways of primary C-oxidations of nicotine in humans

ARE NICOTINE AND/OR COTININE RELEVANT BIOMARKERS FOR CORRELATING ETS EXPOSURE WITH INCREASED INCIDENCE OF LUNG CANCER ?

As indicated above, a relevant biomarker must be unique to the source of exposure. But nicotine, and consequently its metabolite cotinine, are not unique to tobacco smoke. It is present in edible plants of the family of *Solanaceae* (like potatoes, tomatos...) as well as in a number of teas, especially instant tea. Davis *et al.* (29) have calculated a range of potential values for urinary cotinine concentrations (0.6-6.2ng/ml) based upon estimated average and maximal consumption of foods and beverages; nicotine is also consumed as nicotine gums or nicotine dermal patches as well as in chewing or wet snuffing of tobacco. Finally, nicotine is still used as an insecticide in the form of decoctions of tobacco leaf and it is known that nicotine persits in soil. Besides ETS, miscellaneous sources of exposure to nicotine thus exist. They may contribute significantly to the build-up of its metabolite(s) in the plasma, saliva and urine of non-smokers. Because of its short half-life in humans (30-33), nicotine is not a valuable marker. This is the reason why cotinine is classically used to biomonitor exposure to tobacco smoke, including passive smoking. However, large interindividual variations exist in the biodisposition of cotinine, as shown by the sometimes large variabilities in reported urinary excretion (14, 15, 17-19). Moreover this metabolite would be a relevant biomarker for ETS exposure only if it is one of the major end-metabolites of the parent compound. However this is not the case. Indeed, nicotine is extensively metabolized (Fig. 1) via a human cytochrome P450-dependent mixed function oxidase to form the iminium ion which is then oxidised, by a soluble aldehyde oxidase (34, 35), to cotinine (36, 37). This metabolite does not readily accumulate because it is further hydroxylated to 3'-hydroxycotinine (either free or glucurono-conjugated), the major excreted human metabolite of nicotine (38-43). Thus, from a strictly pharmacokinetic point of view, the relevance of cotinine measures for biomonitoring tobacco smoke exposure, particularly at low levels, is questionable. At the very least, studies need to be performed to compare both cotinine and 3'-hydroxycotinine as potential markers for exposure to nicotine.

Another question which also needs to be addressed is the ratio of nicotine (and thus indirectly cotinine or 3'-hydroxycotinine) to other tobacco smoke constituents (for example polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines...). Is this ratio constant enough far measurement to be extrapolated to the other? To meet such a

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requirement, it has to be demonstrated that the ETS to which non-smokers are exposed has the same composition as MS, the reference product for defining potential risk of lung cancer. However, ETS is composed of SS (75-80%) and EMS (20-25%) which are both physically as well as chemically different from MS (2, 3, 8, 44). The main differences between MS and SS, which are relevant to our discussion are attributable to differences [1] in the temperature of combustion of the tobacco (900°C and 600°C respectively), [2] in the pH (6.0-6.7 and 6.7-7.5 respectively), [3] in the degree and the rate of dilution with air, [4] in the size of RSP (0.1-1.0 and 0.01-1.0 microns respectively), and [5] the relative composition of the gas as compared to the particle phase (particularly nicotine, which is predominantly in the particle phase of MS but in the vapor phase of SS). Knowledge of the composition of EMS is very limited and comparative analysis have not been reported. Thus, it is likely that ETS may contain most of the chemicals so far identified in MS. But, as underlined in the US-EPA document (5), "few emission data have been collected under conditions more typical of actual smoking conditions....[and] it is not known how the MS and SS air contaminant emission data for specific compounds generated by standardized testing protocols compare to data gathered under conditions more representative of actual smoking".

The last point concerns the bioavailability of nicotine, which, according to various reports, differs significantly between individuals and in non smokers as compared to active smokers (23, 24, 30). Indeed, high doses of MS induce the enzymes metabolizing not only nicotine but also a wide variety of xenobiotics. This effect is unlikely to occur in non smokers exposed to low doses of ETS.

As a consequence of these differences between MS and ETS, estimates of ETS exposure based on measures of nicotine and/or its metabolite(s) in biological fluids of non-smokers (except perhaps in short-term acute and quite unusual exposure to high dose of ETS) is likely to be misleading, at least in terms of increased lung cancer incidence. Furthermore, because of the differences between ETS and MS as well as because of interindividual variations in biodisposition of nicotine, it is difficult to anticipate whether the values will be over or underestimated (17, 45). A recent paper (46) reports data in guinea pigs exposed to MS or SS which demonstrate that urinary cotinine (ng/mg creatinine) does not correlate with the total amount of inhaled RSP (mg/kg bw/day). In animals exposed to MS or SS from 6 (54 puffs) or 8 (70 puffs) cigarettes, total inhaled RSP were 23 ± 0.7 and 28.3 ± 1.4 mg/kg bw/day respectively after MS exposure, but only 1.5 ± 0.4 and 1.8 ± 0.4 mg/kg bw/day after SS exposure. In the same animals, urinary cotinine (ng/mg creatinine) reached 8.1 ± 2.3 and 10.3 ± 1.8 respectively after inhalation of MS and 3.7 ± 0.15 and 10.6 ± 1.5 respectively after exposure to SS. Based on these data, the calculated ratios between the amount of absorbed RSP and the quantity of urinary cotinine, expressed as mg RSP /kg bw/day per ng cotinine are 2.7 and 2.9 after inhalation of MS but only 0.17 and 0.4 after exposure to SS. Since ETS is mainly composed of SS, such data, if indeed applicable to human exposure, would indicate that an equal amount of urinary cotinine, in MS versus SS (ETS)-exposed individuals, represents quite a different exposure to RSP. Al though they may excrete the same amount of urinary cotinine, SS (ETS)-exposed people might have been exposed to 10 times less RSP than MS-exposed smokers, a conclusion which is consistent with the differences in distribution of nicotine between vapor and particulate phases of MS and ETS.

NEW DIRECTIONS TOWARDS A MORE RELEVANT BIOMONITORING OF ETS EXPOSURE SO AS TO CORRELATE WITH INCIDENCE OF LUNG CANCER IN NON SMOKERS.

For ETS to be considered as a risk factor for lung cancer in non smokers, calculations should be based on defined exposure to relevant chemicals. Neither nicotine nor its metabolites are likely to be precise markers of exposure to such chemicals which are mainly bound to RSP, whereas nicotine is in the vapor phase of ETS. Monitoring of ETS exposure should rely on measurements of bioproducts in physiological fluids which are directly and quantitatively derived from constituents like polycyclic aromatic hydrocarbons, aromatic amines or nitrosamines.... which are mainly in RSP (17, 47). Molecular epidemiology, as it has developed during the last decade, offers a promising, but still to be validated tool to meet such a challenge by measuring proteins and/or DNA adducts in serum or urine samples (48, 49).

Specific and very sensitive analytical methods (50, 51) as well as molecular biology techniques able to identify and quantify modified nucleotides in DNA or modified proteins are presently available, and should be applied to correlate objectively lung cancer with exposure to specific chemicals in non smokers. They are indeed unique in providing the specificity which is required to precisely quantify precisely the low levels of exposure occuring in involuntary absorption of ETS. Such an approach would meet the requirements discussed by Monro (52) under the title "Contemporary issues in Toxicology: What is an appropriate measure of exposure when testing drugs for carcinogenicity in rodents?" By reference to the concept of biologically effective dose introduced by Perrera (53), Monro (52) underlines that "the toxicity of the drug was not reflected by its plasma concentration: instead it depended on the amount converted to a reactive metabolite which then went on to bind with a critical macromolecule" and that "when chemical carcinogenesis is mediated via a reactive metabolite binding covalently to a critical macromolecule ..... the first determinant of the biologically effective dose is that proportion of the administered dose that is converted to the key reactive metabolite. The consequence ... may be also influenced by the rate at which the reactive metabolite becomes available, which in turn may depend on the rate of drug administration...a brief high pulse of reactive metabolite [as it occurs in inhalation of MS] could have different consequences from a prolonged lower delivery rate [as it occurs in ETS exposure] ...". This discussion is relevant to our subject. Indeed, only the evaluation of the biologically effective dose is likely to allow a correct biomonitoring of exposure to be used for conclusively correlating exposure to ETS with any increased incidence of lung cancer. A similar proposal was made by

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Lucier (in: 49) in a symposium held at the 29th annual meeting of the Society of Toxicology. This topic has recently been reviewed extensively in the excellent article of Groopman and Kensler (54) entitled "Molecular bio[illegible] human chemical carcinogen exposures".

One candidate chemical for such an approach is the aromatic amine 4-aminobiphenyl (4-ABP). Because its concentration is higher (≈ 30 times) in SS, it might be a more reliable biomarker for exposure to ETS than nicotine. Metabolic activation and covalent binding to macromolecules, in particular hemoglobin (Hb), yields an adduct. This might serve as a surrogate marker for DNA adducts to evaluate a biologically effective dose (55). Available data, al though still preliminary, show that the methods are indeed adequate to assay the low levels of adducts found in the biological fluids of non smokers (a few pg/g Hb) and that a relationship might exist between exposure to ETS and the levels of 4-ABP-Hb adducts in non smokers (9, 55-58). Such a new experimental approach will require extensive research before being validated to assess real exposure to ETS and to establish a link between such an exposure and increased risk of lung cancer. Among other questions which need to be answered are: [1] the specificity of ETS as a source of 4-ABP; [2] the pharmacokinetics of 4-ABP-Hb adduct formation in human blood and relating its level to the time, duration, frequency of exposure to well defined ETS; [3] the quantitative, if any, relationship between a specific "dose" of ETS and the blood level of the adduct; and [4] the correlation, if any, between the 4-ABP-Hb adduct and other adducts likely to be formed because of exposure to other agents. Indeed 4-ABP is not recognized as a lung but rather as a bladder carcinogen, and there is no convincing epidemiological evidence to suggest that the risk of bladder cancer is increased in non smokers. Since polycyclic aromatic hydrocarbons and in particular benzo(a)pyrene (59), as well as nitrosamines (50), also form adducts, it seems reasonable to at least explore these possibilities.

CONCLUSIONS

ETS is a complex mixture of diluted MS and SS composed of gases and RSP. There is no doubt that people living in the proximity of an active smoker are exposed to and absorb constituents of ETS. However, it is both a highly diluted and an aged mixture, the composition of which is qualitatively and quantitatively different from MS. When epidemiologists work to correlate low levels of ETS exposure with an increased risk of lung cancer they should be advised not to rely only on questionnaire results because of the many biases. Quantitative molecular epidemiology will help to resolve the question of the actual risk of such exposure. However, the relevant marker remains to be chosen. Neither nicotine nor its metabolites fulfil the criteria. Quantitative measurements of molecular adducts to macromolecules might be promising tools, but many basic questions still need to be answered before they can be applied routinely. These basic questions concern not only the analytical aspects, but also the mechanisms of any toxic potential including the cause of large interindividual variations in metabolic capacity for activation and inactivation of carcinogens as well as in repair mechanisms (59) that might explain any increased incidence of lung cancer. Indeed, quantitative molecular epidemiology will contribute to a better correlation between ETS exposure and increased risk of lung cancer in non smokers only if it continues to benefit from progresses in basic research in toxicology. Indeed, as we have discussed repeatedly, to improve the science in toxicology is the necessary key to real progress in risk assessment (59). To correlate the concentration of a particular macromolecular adduct with increased risk of lung cancer in non smokers exposed to ETS will benefit from any data which support the hypothesis of a causal relationship between the *in vivo* concentration of an adduct and the pathogenic process leading to the disease. ■

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Corrélation entre le tabagisme passif et l'incidence accrue de cancers pulmonaires: la cotinine est-elle un marqueur valide ?

RESUME

La fumée du tabac présente dans l'environnement est un mélange complexe de la fumée exhalée par le fumeur et la fumée qui s'échappe du tabac en combustion. Cette fumée est composée de substances volatiles et de particules solides en suspension qui forment un aérosol respirable. Elle est tout à la fois très diluée et vieillie par rapport à une fumée inhalée dont la composition est qualitativement et quantitativement différente. En utilisant deux syllogismes, l'hypothèse a été émise que l'exposition à de faibles doses de fumée de tabac présente dans l'environement augmente le risque de cancer du poumon chez les non-fumeurs. Il est évident que les non-fumeurs qui vivent en présence d'un fumeur sont exposés à certains constituants de la fumée du tabac. Toutefois, pour pouvoir conclure à la signification d'une telle exposition, en terme d'incidence accrue de cancer du poumon chez les non-fumeurs, il importe de disposer des résultats quantitatifs qui démontrent une exposition à des agents connus pour jouer un rôle causal dans la cancérogenèse pulmonaire. Ni la nicotine, ni ses métabolites ne sont des marqueurs précis de l'exposition à de tels agents cancérigènes. L'épidémiologie moléculaire permet d'envisager une approche de cette question en mesurant des adduits aux protéines ou à l'ADN dans le sérum ou les urines. Toutefois, une telle approche doit encore être validée.

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SMOKE: CONCENTRATION AND

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Environmental tobacco smoke (ETS) is often cited as a key factor in indoor air quality (IAQ) and public health. However, there are few studies which attempt to actually calculate the impact ETS has on IAQ or the doses of ETS one may receive from possible exposure in a variety of different settings. This paper reviews the data on indoor air published since 1980 and estimates the portion of various constituents which are produced by ETS. It can be observed that, in most instances, ETS has only a minor impact on IAQ. Retained doses of ETS particles are calculated for various exposure scenarios using respirable particle concentrations associated with ETS, time activity patterns, respiration rates and retention rates. Total doses range from 3-40 mg/y. This dose level does not seem to support the summary relative risk of 1.35 that has been claimed from meta-analyses of epidemiologic studies of spousal smoke exposure and lung cancer.

INTRODUCTION

Interest in indoor air quality (IAQ) is steadily increasing. The factors which affect IAQ and the health effects reportedly associated with it are the subject of intense debate. One of the IAQ issues which generates the most interest and emotion is environmental tobacco smoke (ETS) exposure and its reported health effects. ETS exposure has been claimed to be associated with health effects as diverse as childhood respiratory disease, lung cancer, and cardiovascular disease (Repace and Lowrey 1985; U.S. Surgeon General 1986; National Research Council 1986; Wells 1988; Glantz and Parmley 1991; USEPA 1990).

ETS is a complex mixture of many substances, the concentrations of which will vary with time, room ventilation, and proximity to the source. Since not all of its components are removed from the environment at the same rate, the concentrations of ETS components also vary in relation to each other over time. Because of this, it is difficult to accurately determine exposure to ETS and, further, whether the health claims are realistic in terms of this exposure.

Sterling et al. (1982) performed a comprehensive review of components of ETS measured in different environments and under different smoking conditions. Since that review was completed, there have been many changes in both indoor environments and analytical methodology. Because of this, a new review of the literature pertaining to indoor air quality and environmental tobacco smoke is appropriate.

This study assesses the literature on indoor air quality and ETS published since 1980. Using the data collected, it also attempts to determine what levels

of substances measured indoors may result from the presence of ETS and calculates some of the doses [illegible] be expected from [illegible]

INDOOR AIR AND ETS REVIEW

Methods

The literature search was restricted to work which took place in the U.S. and Canada and was published after 1980. There is important IAQ data being generated in European and other countries. However, potential differences in building age, ventilation types, room sizes and other factors may prevent data from other countries from being comparable to the U.S./Canadian data. This study limited the literature to the countries where the data were believed to be more homogeneous and essentially reviewed the literature published since the Sterling et al. (1982) review.

The following indoor air components were chosen for evaluation: respirable particulates (RSP); carbon monoxide; nicotine; nitrogen dioxide; formaldehyde; benzene; polycyclic aromatic hydrocarbons (PAH); and nitrosamines.

When it was obvious that structures and sampling protocol for data acquired in countries outside North America were similar, it was included with the USA/Canada data. In instances where relatively little information was available or the data from all countries were similar, information from other countries also was used.

The information was recorded in the following categories:

1. Homes—includes single family dwellings and apartments.
2. Offices, workplaces, and public facilities—includes offices, work sites, schools, universities, hospitals, retail stores, museums, libraries, clinics, rocery stores, laundromats, and public transportation stations.
3. Restaurants
4. Bars/taverns—includes betting shops, billiard parlors, bars, and taverns.
5. Public transportation—trains, buses, subway, and autos.

The data were selected from the literature using the following criteria:

If no mean was given, generally, the data were not reported in this document unless there were individual values given to make it possible to calculate a mean. If there were 10 or more samples and a median was given, the data were reported. Both arithmetic and geometric means are reported. If an arithmetic mean was given, it was used in any subsequent calculations.

If only [illegible] the data were not used because one sample at one point in time is not as representative [illegible] several samples at different [illegible] longer duration.

For respirable particulates, if the data were reported as total particulate matter (TPM), the data were not used. If the sample was PM 5.0 or less, the data were reported.

The data were recorded with a preference for gravimetric data on RSPs. When gravimetric, light scattering, and piezobalance data were all present, the gravimetric data were used. If data from only one of these three methods were present, these data were used.

This paper focuses upon the scientific literature pertaining to the quantification of indoor air quality. Hence, the papers reviewed are those that have measured levels of substances in indoor air. Odor may play a part in the acceptability of indoor air to occupants or visitors to any particular environment, but the evaluation of odor in offices is as yet somewhat subjective and poorly quantified. ETS clearly may influence odor perception in some situations, and the existing scientific literature on this matter has been reviewed. Because of both the scarcity of data on this issue and the subjectivity of the data that do exist, odor has not been considered as a quantified element in the data tabulated in this report.

Results

The results of the literature review on indoor air components are in Tables 1-8. Each table is a summary of one of the components reviewed. Units of measurement in the tables are reported the same as authors presented them in their studies. Conversion factors for ppm (parts per million) and ppb (parts per billion) to $\mu g/m^3$ are given where appropriate. Tables 9, 10, and 11 summarize the data for RSPs, CO, and nicotine. Nitrogen dioxide, formaldehyde, benzene, PAH, and nitrosamine data are summarized in the discussions.

DOSIMETRIC CALCULATIONS

Methods

The particle fraction of ETS is the portion on which the majority of the health claims concentrate. An estimate of the dose of ETS particles that persons

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Table 1. Respirable particles (RSPs) measured under realistic conditions.

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations
Carson & Erikson (1988)	Canada	Offices (31)	≥ 2 people w/min. 1 smoker	Not Given	PASS Unit PM3.5 9 hr samples (8-5)	UVPM ($\mu g/m^3$) A. Mean 44 G. Mean 24 Range 6 - 426
Conner, et al. (1986)	USA	Homes (10) Restaurants (10)	Not Given	Not Given	Gravimetric & UVPM Personal Pump, 120-180 min. samples.	RSP ($\mu g/m^3$) / UVPM ($\mu g/m^3$) Homes, Range 17 - 86 / 1 - 8 Homes, A. Mean 58 / 4.2 Rest., Range 18 - 306 / 15 - 223 Rest., A. Mean 169 / 106
Conner, et al. (1989)	USA	Offices (10) Planes (5)	Not Given	Not Given	Gravimetric PM3.5 PASS Unit, 1-5 hr samples	RSP ($\mu g/m^3$) / UVPM ($\mu g/m^3$) Offices, Range 167 - 1088 / nd - 147 Offices, A. Mean 448 / 65 Planes, Range (Smoking Section) 33 - 119 / 20 - 106 Planes, A. Mean (Smoking Section) 72.6 / 66.6 Planes, Range (Non Smoking Sect.) 3 - 98 / 12 - 30 Planes, A. Mean (Non Smoking Sect.) 22 / 18.6
Coultas, et al. (1990a)	USA	Workplace(15)	Not Given	Not Given	Gravimetric Personal Monitors 6.5 hours, PM2.5	Males / Samples / Avg. RSP ($\mu g/m^3$) Hospital 2 66.15 Offices 2 56.7 Barber Shop 2 80.25 Restaurant 1 145.8 Retail Store 1 85.2 Females / Avg. RSP ($\mu g/m^3$) Hospital 3 35.33 Offices 3 70.37 Public Trans. 1 4.0
Coultas, et al. (1990b)	USA	Homes (10)	Not Given	Not Given	Gravimetric PM2.5 10 samples/home	RSP Means = 32.4 - 76.9 $\mu g/m^3$
Cousins & Collett (1989)	Canada	Schools (3) Portables (6)	Not Given	Not Given	Light Scattering PM5.0	RSP ($\mu g/m^3$): Old School / Ren. School / New School / Port. Class. Indoor 16 (11-22) / 13 (10-20) / 14 (10-17) / 17 (15-20) Outdoor 23 / 15 / 18 / 18
Crouse & Carson (1989)	USA	Offices(32) & Restaurants (36)	Not Given	Not Given	Gravimetric PM3.5	Geo. Mean / Range ($\mu g/m^3$) Offices RSP 61 / 11 - 279 UVPM 47 / 11 - 84 Restaurants RSP 111 / 16 - 366 UVPM 31 / 10 - 194
Crouse, et al. (1988)	USA	Restaurants (37)	Not Given	Not Given	Gravimetric PM3.5 1 Hour Sample	Geo. Mean / Arith. Mean / Range ($\mu g/m^3$) RSP 62 ± 2.2 / 80.8 / 16 - 221 UVPM 26.1 ± 1.9 / 34.1 / 15 - 168

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Table 1. Continued.

Daisey, et al. (1989)	USA	Homes (7)	Not Given	0.13-0.89 ACH	Gravimetric w/ Cyclone, 48 hr samples	see below
Eudy, et al. (1987)	USA	Restaurant	Not Given	Not Given	Gravimetric PM3.5 4 hr samples, 12 samples/48 hr run	see below
First, (1983)	USA	Public Places	Various	Not Given	Piezobalance	see below
Georghiou, et al. (1989)	Canada	Ice Arena	Varied	Not Given	PM10 Indoor Sampling Impactor, 2.5 hr samples 10 games	see below
Grimsrud, et al. (1990)	USA	Office Buildings (40)	Not Given	Not Given	Gravimetric PM3.0 3-20 sampling sites/ building	see below
Hedge, et al. (1990)	USA	Office Buildings (2)	Not Given	Not Given	Piezobalance, Gravimetric PM2.5, UVPM	see below

Daisey, et al. (1989):

House #	Indoor	Outdoor	(+ = Woodburning)
102 +	37.5	24	
-	30.4	13.8	
106 +	33.3	30.9	
-	57.7	7.2	
108 +	36.6	38.4	
-	27.6	27	
203 +	19.7	32.4	
-	20.3	4.5	
204 +	35.4	10.5	
-	27.3	9.6	
208 +	47.6	19.9	
-	29.9	-	
300 +	60.1	-	
-	35.6	-	

Mean - Woodsmoke 32.7

Eudy, et al. (1987):

RSP, 5 - 48 hour sample runs

Range 0 - 105 $\mu g/m^3$

A. Mean 29.3 $\mu g/m^3$

First, (1983):

Site	Avg. Conc. ($\mu g/m^3$)
Chamber1	300 - 1 cig. smoked
Chamber2	290 - 1 cig. smoked
School cafeteria1	20 - no smokers
School cafeteria2	40 - 2-3 smokers
Tavern1	400 - 1-5 smokers
Tavern2	660 - 2-3 smokers
Tavern3	570 - mostly 1 smoker
Bus Terminal	110 - 50-100 people, 1-5 smokers
Bus Terminal (outside)	70
Fast Food Restaurant	150 - 1-3 smokers during sampling
Sm. Sitdown Rest.1	250 - 15 diners, 4 smokers
Sm. Sitdown Rest.2	260 - 23 diners, 1 smoker

Georghiou, et al. (1989):

	Range ($\mu g/m^3$)	A. Mean ($\mu g/m^3$)
Smoking Allowed	17 - 680	440.75 (n=4)
Smoking Restricted	187 - 426	302.57 (n=7)

Grimsrud, et al. (1990):

	Concentration ($\mu g/m^3$)
Arithmetic Mean	30.0
Geometric Mean	24.0
Range	5 - 86

Hedge, et al. (1990):

Area	Method	Data ($\mu g/m^3$) AM	PM
Building (NS)	Piezobalance	20	10
Office (Restricted S)	Piezobalance	40	40
Smoking Area w/filter	Piezobalance	110	140
Building (NS)	Gravimetric	N/A	N/A
Office (Restricted S)	Gravimetric	200	300
Smoking Area w/filter	Gravimetric	350	400
Building (NS)	UVPM	0	0
Office (Restricted S)	UVPM	9.0	7.0
Smoking Area w/filter	UVPM	120	185

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Table 1. Continued.

Hollowell and Miksch 1981	USA	Office (1)	Not Given	Not Given	12 Hours	"Particles" - - - 31 µg/m³ Avg.
Hosein, et al. (1985)	Canada	Homes	Not Given	Not Given	Gravimetric (RSP) 24 hr. samples	RSP (µg/m³, Geometric Mean) AC / No AC Smoke (n=11) 80.5 / (n=25) 70.11 No Smoke (n=4) 34.3 / (n=11) 32.5 Carpet / No Carpet Smoke (n=28) 76.6 / (n=8) 70.2 No Smoke (n=9) 38.7 / (n=7) 53.7 Hot Water / Forced Air Smoke 84.8 / 57.1 No Smoke 66.7 / 37.7
IT Corp. (1987)	USA	Restaurants (36) Offices (38)	Varied	Not Given	Gravimetric PM3.5 PASS Unit 1 Hour Samples	RSP (µg/m³) / UVPM (µg/m³) Offices Range nd-375 / nd-108 Offices A. Mean 114 / 28.3 Restaurants Range nd-417 / nd-127 Restaurants A. Mean 120 / 33.2
Leaderer, et al. (1990)	USA	Homes (394)	Not Given	Not Given	Gravimetric PM2.5	Source / n / Suffolk / n / Onondaga (µg/m³) None 30 17.3 45 14.1 W 15 18.1 16 19.1 K 7 22 4 21.2 S 61 49.3 80 36.5 KW 4 19.7 SW 29 38 31 33.9 SK 23 61.4 4 35.3 SKW 6 30.3 4 28.5 Outdoor 19 16.9 36 15.8 W = Woodstove K = Kerosene S = Smokers
Lofroth, et al. (1989)	USA	Tavern (1)	5 - 25, 180 m³ Smoking Allowed	Not Given	Gravimetric TSP & Piezobalance	First Study (3 hr): Indoor / Outdoor; Second Study (4 hr): Indoor / Outdoor TSP 470 / nd / 390 / nd Piezobalance 420 / < 50 / 320 / 40
McCarthy, et al. (1987)	USA	Homes (68S, 13NS)	Not Given	Not Given	Gravimetric (RSP) Personal & Area Samples (24 hr)	Mean / Median / Range (All: µg/m³) Personal (NS) 29.4 / 27.2 / 21.6-39.8 Personal (S) 56.2 / 52.6 / 18.0-116.3 Home (NS) 30.9 / 25.6 / 16.6-77.1 Home (S) 54.6 / 55.6 / --- Personal Samples on Children (8-11 yr old)
Miesner, et al. (1988)	USA	Public Places, Offices (19)	Varied	Varied	Gravimetric PM2.5 3-16 Hours	Mean RSP (µg/m³) NS Areas (n=33) 14 Smoking Areas (N=7) 34.5 Trans. Facility (n=4) 64 (Subways or Bus)

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Table 1. Continued.

Miesner, et al. (1989)	USA	Public Bldgs (21)	Not Given	Not Given	Gravimetric PM2.5	See results below
Miller (1988)	Canada	Office Buildings (2)	Not Given	Not Given	Piezobalance Pre and 1yr Post smoking ban	See results below
Ogden and Maiolo (1989)	USA	Homes (2) Auto (1) Dep't Store (1) Billiard Parlor (1)	Not Given	Not Given	Gravimetric PM3.5 Personal Pumps 2-8 hr samples	See results below
Oldaker, et al. (1990)	USA & Canada	Restaurants & Offices, 4 cities	Min. 2/off. w/ 1 smoker	Not Given	Gravimetric PM3.5 PASS Unit, Sampled during lunch & dinner hours.	See results below
Oldaker, et al. (1988)	USA	Offices & Restaurants	Not Given	Not Given	Gravimetric PM3.5	See results below

Miesner, et al. (1989):

Samples		Range ($\mu g/m^3$)	A. Mean ($\mu g/m^3$)
4	Library (NS)	5.8 - 12.8	9.08
4	High School (NS)	12.5 - 109.4	41.9
2	Museum 1 (NS)	6.5 - 11.0	8.75
2	Museum 2 (NS)	nd - 12.9	6.45
4	Hospital (NS)	14.5 - 23.3	17.35
2	Hospital (S)	20.1 - 52.5	36.3
4	Clinic (NS)	nd - 17.3	9.3
1	Clinic (S)	119.1	119.1
2	Groc. Store (NS)	11.9 - 14.0	12.95
1	Laundromat1 (NS)	17.2	17.2
1	Laundromat2 (NS)	24.3	24.3
2	Bar/Rest.1 (S)	36.3 - 107.3	71.8
2	Bar/Rest.2 (S)	133.1 - 140.9	137
3	Bar 3 (S)	30 - 78.3	59
2	Subway St.1 (NS)	91.7 - 157.3	124.5
2	Subway St.2 (NS)	55.1 - 66.5	60.8
1	Bus Station (NS)	43.3	43.3
2	Office Bdg1 (S)	16.2 - 18.6	17.4
1	Office Bdg2 (NS)	15.8	15.8
2	Office Bdg3 (NS)	17.3 - 18.2	17.75
3	Office Bdg4 (S)	26 - 80	44.73
2	Office Bdg4 (NS)	15 - 15.2	15.1
1	Office Bdg5 (S)	520.8	520.8
4	Office Bdg5 (NS)	11.1 - 20	14.83
2	University (S)	114 - 196	155
2	University (NS)	5.6 - 44.3	24.95

Miller (1988):

Floor	Building A	Floor	Building B
7th Pre	30 $\mu g/m^3$	3rd Pre	35 $\mu g/m^3$
9th Pre	28	15th Pre	47
7th Post	22	3rd Post	18
9th Post	22	15th Post	25

Ogden and Maiolo (1989):

	Sample Duration	No. of Cigs.	Conc. ($\mu g/m^3$) RSP	Solanesol
Billiard Parlor	2 hr	34[a]	355	12.8
Home	4 hr	6[b]	187	6.4
Home	4 hr	6[b]	212	6.6
Dep't Store	4 hr	0	55	ND
Automobile	8 hr	0	18	ND

[a]Actual Count, 30 cigs., 4 cigars.
[b]Kitchen only.

Oldaker, et al. (1990):

	Offices ($\mu g/m^3$)	Restaurants ($\mu g/m^3$)
RSP Range	(n=131) 0 - 1,088	(n=83) 0 - 685
RSP Mean	126	126
UVPM Range	(n=125) 0 - 287	(n=82) 0 - 184
UVPM Mean	27	36

Oldaker, et al. (1988):

		RSP ($\mu g/m^3$)	UVPM ($\mu g/m^3$)
Offices (n=45)	GM	95	(n=43) 24
	AM	107	33
	Range	7-258	2-170
Restaurant (n=47)	GM	175	(n=49) 47
	AM	199	61
	Range	57-658	7-163

Table 1. Continued.

Ozkaynak, et al. (1990)	USA	Homes (9)	18 participants	0.2 - 0.8 ACH	Gravimetric PM2.5 Stationary & Personal Samples

	N	Average	St. Deviation	Range - $\mu g/m^3$
Indoors	230	37.31	24.27	6.97-168.82
Personal	52	75.09	46.82	19.90-240.56
Outdoor	101	42.72	23.97	8.98-116.87

Proctor (1989)	UK	Offices (10) Train Comp.(20)	app. 75%	Mechanical	Gravimetric PM3.5 PASS Unit, 1 Hr Samples

($\mu g/m^3$)	RSP Range	RSP Mean	UVPM Range	UVPM Mean
Offices(S)	33-260	103	.5-75	23
Office(NS)	29-240	90	1-17	8
Trains (S)	70.8-325	216	13-110	59.8
Trains (NS)	63.3-450	186	9-105	33

Proctor, et al. (1989b)	UK	Office (1)	26 m^2/ person	Not Given	Gravimetric PM3.5 10 sites sampled 5 times each

	RSP ($\mu g/m^3$)	UVPM ($\mu g/m^3$)
S Mean -	103	23
Median -	91	24
NS Mean -	90	8
Median -	71	8.8

Quackenboss, et al. (1989a)	USA	Homes (98)	Not Given	Not Given	Gravimetric PM10 & PM2.5

Smoking	Evap. Cool.	n	PM2.5 ($\mu g/m^3$)	n	PM10 ($\mu g/m^3$)
	Yes	20	8.8	20	21.0
No	No	25	20.3	23	38.4
	Total	45	15.2	43	30.3
	Yes	10	19.3	10	33.9
1-20/day	No	16	32.3	17	53.4
	Total	26	27.3	27	46.2
	Yes	8	36.2	9	47.4
>20/day	No	9	82.7	9	102.5
	Total	17	60.8	18	75.0

Quackenboss, et al. (1991)	USA	Homes (200)	Not Given	Not Given	Gravimetric PM2.5 2 weeks of sampling

	Smokers at Home		No Smokers at Home	
Season[a]	N	Median $\mu g/m^3$	N	Median $\mu g/m^3$
Summer	49	20.5	50	8.9
Spring/Fall	39	20.1	37	10.6
Winter	24	35.7	26	13.4

[a]Seasons: Summer = May - September
Spring/Fall = March, April, October, November
Winter = December - February

Santanam, et al. (1990)	USA	Homes (280) 70S, 70NS each city	Not Given	Not Given	Gravimetric PM2.5 1 week samples

Steubenville (RSP $\mu g/m^3$)

Winter		Summer	
S Homes	NS Homes	S Homes	NS Homes
43.57	19.54	49.85	29.5

Portage (RSP $\mu g/m^3$)

Winter		Summer	
S Homes	NS Homes	S Homes	NS Homes
34.6	14.8	24.9	13.9

Sheldon, et al. (1988a)	USA	Home for Elderly (2)	Not Given	Not Given	Dichotomous Impactors < 2.5μm

RSP <2.5μm ($\mu g/m^3$) Mean of 3 - 24 Hour Samples

	Home #2	Home #1
Apartment (S)	89	39
Commons Area	16 (smoking observed)	30 (smoking lounge)
Apartment (NS)	9	9
Outdoors	4 (1 24 hr sample)	10

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Table 1. Continued.

Spengler, et al. (1985)	USA	Homes Offices Workplace (Non-office)	Not Given	Not Given	Gravimetric PM2.5	Building Type / Smoking ($\mu g/m^3$) / Non-smoking ($\mu g/m^3$) Homes: N = 80 / 186; A. Mean 74 / 28 Offices: N = 31 / 80; A. Mean 67 / 24 Workplace (Non-office): N = 9 / 6; A. Mean 50 / 23
Spengler, et al. (1987)	USA (6 cities)	Homes App. 300	Not Given	Not Given	Particle Sampler PM2.5 Week Long Samples	Range of Means - Homes Non Smoking 15 - 35 $\mu g/m^3$ Smoking 35 - 75 $\mu g/m^3$ Homes w/smokers 30$\mu g/m^3$ > Homes w/o smokers (Mean)
Sterling & Mueller (1988)	Canada	Office Building (2)	Not Given	Mechanical/ Re-circulated Air Pass. Vent/No Re-circulation	Light Scattering, Digital Dust Counter < 5 μm	# Samples / RSP ($\mu g/m^3$) Cafeteria (S) 6 / 102.5 Cafeteria (NS) 6 / 64.5 Offices (NS) 8 / 6.0 Offices (NS) 2 / 7.0
Sterling, et al. (1988)	USA & Canada	Offices	Not Given	Not Given	?	"Particles" ND - 2.7 mg/m^3 (0.04 median) 85 samples
Thomas, et al. (1989)	USA	Industrial Cafeteria	30 ± 9 560 m^3	8.8 ACH 2900 cfm OA, 2500 cfm RA	Gravimetric PM3.5 2 hrs/day for 14 Days (Lunchtime)	Lunchtime ($\mu g/m^3$) / Background ($\mu g/m^3$) RSP 60 ± 50 / 10 ± 10 UVPM 54 ± 33 / < 1 (Avg. Smoking Rate During Sampling was 26 ± 6 cig/hr)
Turner, et al. (1991)	USA	Offices (585)	126.5 ft^2/ person	Not Given	Piezobalance	N / RSP ($\mu g/m^3$) means Smokers 331 / 46.37 Non smokers 254 / 20.11 Total 585 / 34.97
Weschler and Shields (1989)	USA	Phone Switching Office (1)	Not Given	Not Given	Gravimetric PM2.5 1 week samples	Week / Indoors ($\mu g/m^3$) / Outdoors ($\mu g/m^3$) Apr. 7-14 0.85 / 7.41 Apr. 14-21 1.22 / 10.33 Apr. 21-28 3.05 / 17.85 Apr. 28 - May 5 0.67 / 7.80 May 5-12 3.50 / 16.62 May 12-19 4.12 / 17.59 May 19-26 2.41 / 12.40 May 26 - June 2 3.12 / 20.24
Yocom, (1982)	USA	School, Homes (1) (2)	Not Given	(See Results)	Resp. Part. Matter PM2.5	RPM @ Elementary School ($\mu g/m^3$) Vent. (m^3/h/person) / Indoors / Outdoors 10.8 / 19.0 / 26.3 9.1 / 11.7 / 15.3 2.5 / 15.5 / 26.3 RPM in Home Pre & Post Energy Efficient Retrofit Pre Indoor / Pre Outdoor / Post Indoor / Post Outdoor Home 1, S 31 / 10 / 36 / 11 Home 1, NS 9 / 14 / 8 / 13 Home 2, NS 12 / 9 / 10 / 10

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Table 2. Carbon monoxide (ppm) measured under realistic conditions (Conversion factor: 1ppm = 1.15 mg/m³).

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Carson & Ericson (1988)	Canada	Offices (31)	≥2 people w/min. 1 smoker	Not Given	Electrochemical Detector in PASS Unit, 1 sample/ minute	Indoor / Outdoor (ppm) A. Mean 1.9 / 1.9 G. Mean 1.3 / 1.2 Min. <.1 / <.1 Max. 8.7 / 5.8 No. of Offices 28 / 21
Cousins & Collett (1989)	Canada	Schools (3)	Not Given	Not Given	Electrochemical Analyzer	CO (ppm): Old School / Renovated School / New School / Portables A. Mean 1.3 / 1.1 / 0.9 / 0.9 Range 1.0-1.8 / 0.7-1.5 / 0.7-1.1 / 0.8-1.0 Outdoor 2.1 / 11.5 / 1.1 / 1.1
Crouse, et al. (1989)	USA	Offices (30) Restaurants (30)	Not Given	Not Given	PASS Unit, Electrochemical Detector	N / Range (ppm) / A. Mean (ppm) Offices-Outdoors 29 / 0.2-7.2 / 2.8 Offices-Indoors 29 / 0.6-7.1 / 2.5 Restaurants-Outdoors 31 / 0.2-9.2 / 3.4 Restaurants-Indoors 31 / 0.4-12 / 5.0
Crouse, et al. (1988)	USA	Restaurants (36)	Not Given	Not Given	PASS Unit	N / Range / G. Mean / A. Mean / St. Deviation CO (ppm) 36 / 0.9 - 6.3 / 2.4 / 2.6 / 1.5
Eudy, et al. (1987)	USA	Restaurant	Not Given	Not Given	Electrochemical Analyzer, Sample every 10 mins. during 48 hr runs	CO (5) 48 Hour Sampling Runs Range 0 - 16 ppm A. Mean 4 ppm
First (1983)	USA	Public Places	Various	Not Given	Ecolyzer	Site / Avg. Conc. (ppm) Chamber1 2.0 - 1 cig. smoked Chamber2 1.5 - 1 cig. smoked School cafeteria1 1.0 - no smokers School cafeteria2 0.5 - 2-3 smokers Tavern1 8.0 - 1-5 smokers Tavern2 8.0 - 2-3 smokers Tavern3 7.0 - mostly 1 smoker Bus Terminal 3.5 - 50-100 people, 1-5 smokers Bus Terminal (outside) 2.0-2.5 Fast Food Restaurant 5.0 - 1-3 smokers during sampling Sm. Sitdown Rest.1 6.0 - 15 diners, 4 smokers Sm. Sitdown Rest.2 6.5 - 23 diners, 1 smoker
Flachsbart, et al. (1987)	USA	Car (8) Bus (4) Train (3)	Not Given	Not Given	Electrochemical Detector No Smoking	N[a] / Range of Means (ppm) / A. Mean of means (ppm) Automobile 213 / 8.8 - 22.3 / 11.6 Bus 35 / 3.7 - 10.2 / 6.0 Train 8 / 2.0 - 5.2 / 2.88 [a]N=number of trips
Hedge, et al. (1990)	USA	Office Building (2)	Not Given	Not Given	Direct Reading Interscan 4000 Hourly Samples	A. Mean (ppm): AM / PM Smoking Prohibited 0.0 / 0.0 Smoking Restricted (Office) 2.5 / 1.7 Smoking Restricted (Office with des. smoking area w/LACS[a]) 2.8 / 2.6 [a]Local Air Cleaning System

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Table 2. Continued.

IT Corp. (1987)	USA	Offices (38) Restaurants (36)	Varied	Not Given	Electrochemical Detector, sampled each min. for 60 min.	Offices / Restaurants Range (ppm): 0.5 - 6 / 0.4 - 9 A. Mean (ppm): 1.9 / 3.1
Lofroth, et al. (1989)	USA	Tavern	5-25 people 180 m^3	Not Given	Electrochemical Detector	First Study (3hr) / Second Study (4hr) Indoor: 4.4 mg/m^3 / 4.8 mg/m^3 Outdoor: < 1 mg/m^3 / 1-2 mg/m^3
Mumford, et al. (1990)	USA	Mobile Homes	Not Given	0.5 ACH	SPE CO Detectors	N[a] / Kerosene Heat On / N / Kerosene Heat Off A. Mean: 30 / 7.4 ppm / 37 / 1.5 ppm Maximum: 30 / 11.5 ppm / 37 / 1.5 ppm [a]N=Number of sampling days or number of samples analyzed.
Proctor, (1989)	UK	Offices (10) Train Comp.(20) (10S, 10NS)	Not Given app. 75%	Mechanical Not Given	Electrochemical Detector, 1 sample/minute	(ppm) / CO Range / CO A. Mean (ppm) Offices(S): 0.5 - 5 / 1.4 Office(NS): 0.7 - 4 / 1.2 Trains (S): 1 - 2.2 / 1.6 Trains (NS): 0.5 - 2.9 / 1.3
Proctor, et al. (1989a)	UK	Betting Shops (6)	Not Given	Not Given	Electrochemical Detector	N / Range (ppm) / A. Mean Indoor Smoking: 11 / 3.6-9.4 / 5.1 Indoor N-Smoking: 2 / 2.8-3.4 / 3.1 Outdoor: 16 / 2.8-9.0 / 4.4
Proctor, et al. (1989b)	UK	Office Building (1)	26 m^2/person	Not Given	Electrochemical Detector, five 1 hr continuous samples	Smoking Mean - 1.4 ppm Median - 1.1 ppm Non smoking Mean - 1.2 ppm Median - 1 ppm
Sterling & Mueller (1988)	Canada	Office Buildings (2)	.73-1.8/10m^2	1)Mechanical Air Recirc. 2)Natural, No Recirc.	Electrochemical Analyzer, 3-4 min. samples, 6/location	Building(s) / A. Mean (ppm) Smoking Cafeteria: 1&2 / 3.9 Non Smoking Cafeteria: 1&2 / 2.6 Non Smoking Offices: 1 / 1.8 Non Smoking Offices: 2 / 1.35
Sterling, (1988)	Canada	Office Building	.79/10m^2	Forced Air; recirculation Min. 20 cfm/ person fresh air	Electrochemical Analyzer, 3-4 min. samples, 3/location	A. Mean (ppm) Smoking Prohibited: 2.1 Smoking Permitted: 2.5 Designated Smoking: 4.2
Sterling, et al. (1988)	North Am.	Offices	Not Given	Not Given	Not Given	N / Range / Median (ppm) CO (ppm): 241 / ND - 245 / 2.65
Thomas, et al. (1989)	USA	Industrial Cafeteria	30±9 people 560 m^3	8.8 ACH 5,400 CFM	Electrochemical Sensor, 120 samples/day for 12 days	Average Concentration (ppm) Lunchtime: 0.9 ± 0.4 Background: 0.6 ± 0.2
Turner, et al. (1991)	USA	Offices (585)	126.5 ft^2/ person	Not Given	Electrochemical Detector, 10 readings/hour.	N / CO (ppm) A. Mean Smoking: 331 / 3.40 Non Smoking: 254 / 3.13 Total: 585 / 3.29
Yuill & Comeau (1989)	Canada	Homes (76)	Not Given	0.29 ACH (0.0-1.35)	CO instrument	N / A. Mean (ppm) / Range (ppm) Living Room: 76 / 0.7 / 0.0 - 4.0 Basement: 69 / 0.7 / 0.0 - 4.0 Bedroom: 73 / 0.4 / 0.0 - 3.0

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Table 3. Nicotine measured under realistic conditions.

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Chuang, et al. (1988)	USA	Homes (8)	Not Given	Not Given	Pump w/XAD-4 Sorbent 2 - 8 hr samples in living room	Home #: 1NS, 2S, 3S, 4S, 5S, 6S, 7NS, 8NS, S, NS Nicotine ($\mu g/m^3$): 0.17, 15, 1.7, 29, 45, 4.1, 0.02, 0.06, 19, 0.08
Coultas, et al. (1990a)	USA	Workplace (15)	Not Given	Not Given	Personal Pump w/ Sodium bisulfate filter, 6.5 hr samples	Males — n — A. Mean ($\mu g/m^3$) Hospital 2 28 Offices 2 4.85 Barber Shop 2 8.85 Restaurant 1 45 Retail Store 1 6.9 Females Hospital 3 22.7 Offices 3 33.97 Public Trans. 1 0.0
Coultas, et al. (1990b)	USA	Homes (10)	Not Given	Not Given	Pump w/Sodium Bi-sulfate filter, 10 samples/home	Mean Range 0.6 - 6.9 $\mu g/m^3$ (100 samples)
Crouse, et al. (1988)	USA	Restaurants (37)	Not Given	Not Given	PASS Unit w/XAD-4 Sorbent, 1 hr sample	Restaurants: n 37; Range ($\mu g/m^3$) 1.6 - 34.7; A. Mean ($\mu g/m^3$) 8.6; G. Mean ($\mu g/m^3$) 5.3 ± 2.4
Crouse & Carson (1989)	USA	Offices (32) Restaurants (36)	Not Given	Not Given	PASS Unit w/XAD-4 Sorbent	Offices ($\mu g/m^3$): G. Mean 3.8; Range 1.2 - 24.3 Restaurants ($\mu g/m^3$): G. Mean 4.1; Range 1.0 - 36
Crouse & Oldaker (1990)	USA	Restaurants (21)	Not Given	Not Given	PASS Unit & Personal Pump w/XAD-4 Sorbent Min. 1 hr sample for four months.	PASS Unit: Mean ($\mu g/m^3$) 6.3; Median 4.2; Range 0.3 - 24.8 Personal Pump: Mean ($\mu g/m^3$) 4.3; Median 2.9; Range 0.3 - 24
Eudy, et al. (1987)	USA	Restaurant	Not Given	Not Given	Pump w/XAD-4 Sorbent (12) 4 hr samples during (5) 48 hr sample runs.	Nicotine ($\mu g/m^3$): Range 0.29 - 11.5; A.Mean 2.1
First (1983)	USA	Public Places	Various	Not Given	Pump w/Potassium bisulfate filter	Site — Avg. Conc. ($\mu g/m^3$) Chamber1 13.9 - 1 cig. smoked Chamber2 13.9 - 1 cig. smoked School cafeteria1 5.5 - no smokers School cafeteria2 2.7 - 2-3 smokers Tavern1 6.3 - 1-5 smokers Tavern2 9.4 - 2-3 smokers Tavern3 15.9 - mostly 1 smoker Bus Terminal ---- - 50-100 people, 1-5 smokers Fast Food Restaurant 30.0 - 1-3 smokers during sampling Sm. Sitdown Rest.1 12.0 - 15 diners, 4 smokers Sm. Sitdown Rest.2 16.3 - 23 diners, 1 smoker
Henderson, et al. (1989)	USA	Homes 15 w/Smokers 12 w/o Smokers	Not Given	Not Given	Pump w/Sodium Bisulfate Filter 5pm-7am @ 2 days	Average Concentration ($\mu g/m^3$) Smoking Homes 3.74 Non smoking Homes 0.34

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Table 3. Continued.

IT Corp. (1987)	USA	Offices (38) Restaurants (36)	Not Given	Not Given	Pump w/XAD-4 Sorbent 1 Hr. Samples	Offices ($\mu g/m^3$) / Restaurants ($\mu g/m^3$) Range: nd - 30 / nd - 12 A. Mean: 6 / 4 (Offices & Restaurants sampled had no smoking restrictions)
Jenkins, et al. (1988)	USA	Restaurants (36)	Not Given	Not Given	Personal Pump w/ Tenax Sorbent. 1 hr samples	Nicotine ($\mu g/m^3$) Range: 0.5 - 37.2 A. Mean: 5.78
Lofroth, et al. (1989)	USA	Tavern	5 - 25 $180m^3$	Not Given	Pump w/ filter 3 & 4 hr Samples	1st Study (3hr): 71 $\mu g/m^3$ 2nd Study (4hr): 60 $\mu g/m^3$
McCarthy, et al. (1987)	USA	Homes (81) 68S, 13NS	Not Given	Not Given	Pump w/ Sodium bisufate filter, Personal & Area Samples	Mean / Median / Range ($\mu g/m^3$) Personal (NS): 0.3 / 0.2 / 0.0 - 1.0 Personal (S): 2.5 / 1.9 / 0.1 - 12.0 Home (NS): 0.1 / 0.0 / 0.0 - 1.6 (Personal samples done on children 8-11 yrs old)
Miesner, et al. (1989)	USA	Public Buildings (11)	Not Given	Not Given	Pump w/Sodium bisulfate filter	n / A. Mean ($\mu g/m^3$) Bar (S): 3 / 3.7 Bar/Rest.1 (S): 2 / 7.55 Bar/Rest.2 (S): 2 / 7.85 Subway St. (NS): 1 / 1.0 Hospital (S): 1 / 1.6 Clinic: 1 / 17.1 (Des. Smoking Area in NS Building) Office Bldg1 (S): 1 / 0.6 Office Bldg2 (NS): 1 / 2.0 (Smoking room directly below) Office Bldg3 (NS): 1 / 0.4 Office Bldg4 (S): 2 / 3.15 Office Bldg5: 1 / 26.5 (Des. Smoking Area in NS Building)
Oldaker, et al. (1988)	USA	Offices (46) Restaurants (49)	Not Given	Not Given	Pass Unit w/XAD-4 Sorbent	Offices ($\mu g/m^3$) / Restaurants ($\mu g/m^3$) G. Mean: 3.3 / 4.5 A. Mean: 4.3 / 6.2 Range: 1.0 - 16.3 / 0.7 - 15.6
Oldaker, et al. (1990)	USA & Canada	Offices & Restaurants (4 Cities)	Min. 2 people/office 1 smoker	Not Given	PASS Unit w/XAD-4 Sorbent Tube.	Offices ($\mu g/m^3$) / Restaurants ($\mu g/m^3$) Range: (n=156) 0 - 69.7 / (n=170) 0 - 23.8 Mean: 4.8 / 5.1
Proctor (1989)	UK	Offices (10) Train Compartments (20)	Varied 75%	Mechanical Not Given	PASS Unit w/XAD-4 Sorbent, 1 hr samples.	Offices (S) / Offices (NS) / Train (S) / Train (NS) ($\mu g/m^3$) Range: 0.6 - 26 / 0.1 - 2.1 / 0.6 - 49.3 / 0.5 - 21.2 A. Mean: 6 / 0.6 / 15.3 / 4.5
Proctor, et al. (1989a)	UK	Betting Shops (6)	Not Given	Not Given	PASS w/Tenax Sorbent	n / Range ($\mu g/m^3$) / A. Mean ($\mu g/m^3$) Smoking: 11 / 3 - 57 / 19.36 Non Smoking: 2 / 0.4 - 2 / 1.2 Outdoor: 3 / 0.3 - 0 .4 / 0.33
Proctor, et al. (1989b)	UK	Office (1)	265 ft^2/ person	Not Given	Pump w/XAD-4 Sorbent	Mean ($\mu g/m^3$) / Median ($\mu g/m^3$) Smoking: 6 / 3.1 Non Smoking: 0.6 / 0.6

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Table 3. Continued.

Schenker, et al. (1987)	USA	RR Workers Office, Repair Shop, Outdoors	Not Given	Not Given	Personal Pumps w/Sodium Bisulfate Filter (2 Days)	Mean Concentration ($\mu g/m^3$) Office (n=12) 10.2 ± 2.2 Repair (n=13) 5.8 ± 3.4 Outdoor (n=73) 0.4 ± 0.1
Sterling et al. (1988)	USA & Canada	Offices (32)	Not Given	Not Given	Not Given	Range ND - 43.7 $\mu g/m^3$ Median - ND
Sterling & Mueller (1988)	Canada	Office (1) N-Smoking	.79/10m^2	Forced Air; Recirculation from (S) area	Personal Pump w/XAD-4 Sorbent, 2-8 hr Samples	Range ($\mu g/m^3$) nd - 1
Thomas, et al. (1989)	USA	Industrial Cafeteria	30 ± 9 560 m^3	8.8 ACH 2900 cfm OA 2500 cfm RA	Pump w/XAD-4 Sorbent 2 hr. samples for 14 days (11:30-1:30)	Lunchtime Background Avg. Conc. ($\mu g/m^3$) 5.1 ± 1.6 0.14 ± 0.03 (Average smoking rate during sampling was 26±6 cig/hr)
Turner, et al. (1991)	USA	Offices (585)	126 ft^2/ person	Not Given	Personal Pump w/XAD-4 Sorbent, 1 hour samples.	Nicotine- A. Mean ($\mu g/m^3$) Smoking 6.6 Non Smoking 0.17 Total 3.84
Vaughan & Hammond (1990)	USA	Office	Not Given	Not Given	Passive (M-F) & Active (W) on sodium bisulfate filters.	A.Mean Pre Smoking Policy ($\mu g/m^3$) Post Smoking Policy NS desks (n=13) 2.45 0.3 Snack Bar (n=3) 11.3 85.4 (Designated Smoking) Cafeteria (n=6) 4.5 5.3 S Desks (n=6) 10.7 ----

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Table 4. Nitrogen dioxide measured under realistic conditions (ppm = 1 900 µg/m³; ppb = 1.9 µg/m³).

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Berwick, et al. (1989)	USA	Homes (72)	Not Given	Not Given	Passive tubes, 3 locations/house, 2 week samples	NO_2, µg/m³

	1+2		1 only		2 only		None	
Room	N	NO_2	N	NO_2	N	NO_2	N	NO_2
Kitchen	6	98.50	49	41.07	13	40.92	4	6.40
Living Room	6	76.00	49	43.40	13	24.85	4	6.23
Bedroom	6	104.75	49	38.33	13	28.54	4	5.19
House Ave.	6	90.08	49	40.93	13	31.43	4	5.94

1 = Kerosene heater 2 = Gas stove

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Brauer, et al. (1990)	USA	Homes (11)	Not Given	Not Given	Passive samplers Triethanolomine Colorimetric 24 hr. indoor, 48 hr. outdoor	NO_2, ppb

	N	Mean	Min.	Max.
Indoor	30	17	7	36
Outdoor	30	15	5	26

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Dumont, (1986)	Canada	Homes (46)	Not Given	Varied	Passive samples, 1 week, 1 sample/house	

NO_2	N	A. Mean (ppb)
Wood stove, smoke	9	5.6
Wood stove, no smoke	21	5.1
No wood stove, smoke	4	5.3
No wood stove, no smoke	12	3.5

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Good, et al. (1982)	USA	Homes (90)			Palmes personal sampler, 7 day, kitchen, betroom, living room.	NO_2, means, µg/m³

Summer	S home	NS home
Avg. No. Smokers	2.2	2.7
Avg. No. NSmokers	1.3	0.3
Winter	**S home**	**NS home**
Avg. No. Smokers	2.0	2.7
Avg. No. NSmokers	1.4	0.2

vent. rates not given

Stove	# of		Kitchen		Living Room		Bedroom	
	Cig.	N	Sum.	Win.	Sum.	Win.	Sum.	Win.
Elec.	> 20		15.6	21.3	16.5	23.5	14.0	21.3
Gas	> 20	8	76.3	156.6	66.9	112.2	48.4	96.4
Elec.	≤ 20		11.8	20.3	12.4	19.6	10.7	17.5
Gas	≤ 20	3	87.0	219.6	47.1	117.4	38.7	97.8

NO_2, µg/m³

Room	Season	Smoke	N	Mean	Max	Min
Living	Summer	NS	54	12.4	86.5	-2.5
Room		S	38	16.5	40.8	-0.4
	Winter	NS	49	17.5	36.6	5.7
		S	38	21.3	49.6	-1.6
Bedroom	Summer	NS	54	10.7	66.9	-2.7
		S	38	14.0	42.7	-0.6
	Winter	NS	50	20.3	57.1	5.3
		S	38	21.3	54.3	1.4
Kitchen	Summer	NS	54	11.8	72.0	-3.4
		S	38	15.6	44.7	0.5
	Winter	NS	49	19.6	44.5	5.7
		S	38	23.5	65.6	1.4
Outside	Summer	NS	54	21.3	70.5	1.1
		S	38	22.6	54.5	6.3
	Winter	NS	48	52.3	99.9	18.7
		S	35	50.0	91.3	8.9

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Hollowell and Miksch (1981)	USA	Office (1)	Not Given	Not Given	One week	NO_2 - 60 µg/m³, 30 ppb

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Table 4. Continued.

Hosein, et al. (1985)	Canada	Homes (52)	Not Given	Not Given	NaOH impinger	NO_2 μg/m^3, Geometric means (see below)
Marbury, et al. (1988)	USA	Homes (144)	Not Given	Not Given	Passive sample tubes 2, 2 week samples, 3 samples/home	NO_2, means and range, ppb (see below)
Morey and Jenkins (1989)	USA	Offices (7) (problems reported)	Not Given	Not Given	Triethanolamine tube 50-200 ml/min., colorimetric	NO_2, ppm (see below)
Moschandreas, et al. (1990)	USA	Homes (18) w/gas furnaces	Not Given	Not Given	Portable Chemi-luminescence det., 4 samples ≤ 15 min.	(see below)

Hosein, et al. (1985): NO_2 μg/m^3, Geometric means

	Air Cond. N	Air Cond. NO2	No Air Cond. N	No Air Cond. NO2
Gas Stove	4	175.1	7	182.1
Elec. Stove	12	70.9	29	81.8
No Gas	Summer N	Summer NO2	Winter N	Winter NO2
Smoke	29	76.1	29	82.6
No Smoke	12	74.5	12	75.7

Marbury, et al. (1988): NO_2, means and range, ppb

	Gas stove 1	Gas stove 2	Elec. stove 1	Elec. stove 2
Outside	19.1 (5.2-26.7) N=36	20.3 (6.9-31.6) N=38	14.1 (5.1-24.3) N=38	19.6 (5.1-30.1) N=28
Activity Room	41.3 (8.4-168.7) N=81	39.3 (7.0-135.9) N=74	7.8 (2.0-20.9) N=59	7.0 (1.3-22.7) N=53
Bedroom	33.1 (4.4-167.1) N=82	30.9 (4.0-140.4) N=75	7.0 (1.6-32.5) N=60	6.2 (1.1-22.4) N=56

1 = 1st cycle of samples 2 = 2nd cycle of samples

Morey and Jenkins (1989): NO_2, ppm

Bldg.	Outdoor, roof	Indoor
E	<0.07	<0.06-0.10
E	----	0.2-0.3
F	0.04	0.20
G	0.05	0.09-0.10
H	0.10	<0.07-0.09
I	0.10	0.03-0.16
J	<0.02	<0.02-0.20
K	<0.02-0.05	<0.03-0.60
K	0.03-0.08	0.04-0.70

Moschandreas, et al. (1990):

NO_2	Appliance Room (ppb) Mean	Appliance Room (ppb) Range	Control Room (ppb) Mean	Control Room (ppb) Range
Furnace Off	29	3 - 33	32	7 - 40
Furnace On	40	3 - 58	42	4 - 54

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Table 4. Continued.

Petreas, et al. (1988)	USA	Mobil Homes (312)	Not Given	Not Given	Palmes tubes, 3 sites/house, one week samples

NO_2, ppb

		Room	N	A. Mean	G. Mean
Summer, all		Kitchen	311	20.73	16.92
		Bedroom	312	14.64	11.71
		Outdoor	30	11.64	8.95
	gas	Kitchen	263	22.89	20.25
		Bedroom	265	15.99	13.51
		Outdoor	25	11.20	8.39
	elec.	Kitchen	47	8.98	6.18
		Bedroom	46	7.77	5.14
		Outdoor	5	13.78	12.33
Winter, all		Kitchen	254	26.56	21.09
		Bedroom	254	18.67	15.10
		Outdoor	27	22.61	19.16
	gas	Kitchen	230	28.68	24.33
		Bedroom	230	19.98	16.77
		Outdoor	23	22.41	18.77
	elec.	Kitchen	23	6.29	5.36
		Bedroom	23	6.07	5.48
		Outdoor	4	23.74	21.52

gas = gas stove elec. = electric stove

			N		
Summer	Kitchen	LA	61	36.92	24.26
		Non-LA	250	19.26	15.66
	Bedroom	LA	60	22.31	19.44
		Non-LA	252	12.97	10.27
Winter	Kitchen	LA	46	34.75	30.52
		Non-LA	208	24.76	19.55
	Bedroom	LA	48	25.74	21.26
		Non-LA	206	17.03	13.98

LA = Los Angeles basin Non-LA = Not in LA

Spengler, et al. (1987)	USA	Homes	Not Given	Not Given	Palmes Passive Sampler

NO_2	Gas Home (ppb)			Electric Home (ppb)		
	N	Mean	SD	N	Mean	SD
Fall	160	27.6	10	68	11.8	2.5
Summer	142	25.2	6.6	68	14.4	2.5
Winter	166	24.3	5.4	70	17.1	3.5

Sterling, et al. (1988)	USA & Canada	Offices	Not Given	Not Given	Not Given

	N	Median	Min.	Max.
NO_2	49	ND	ND	0.1 ppm
NO_x	40	ND	ND	26.3 ppm

Yocom, (1982)	USA	Homes (9)	Not Given	Not Given	Palmes tubes, 4 week; Mean values ($\mu g/m^3$)

NO_2		Gas stove (5)	Elec. Stove (4)
Fixed,	Outside	17.1 $\mu g/m^3$	16.9 $\mu g/m^3$
	Kitchen	59.2	15.7
	Bedroom	37.3	14.5
Personal,	Husband	36.3	19.8
	Wife	40.8	16.7
	Child	45.2	8.7

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Table 5. Formaldehyde measured under realistic conditions (ppm = 1 230 μg/m³).

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Dumont, (1986)	Canada	Homes (46)	Not Given	0.21 ach, ave.	Passive badges 2 samples/house 1 week/sample	Formaldehyde (ppm) Mean / Median / Range 0.097 / 0.091 / 0.03-0.24
Girman, et al. (1989)	USA	Office Building (52,000 sq. ft.)	Not Given	Mechanical	Midget Impingers chromotropic acid analysis, formaldehyde	Pre-Bake Out ($\mu g/m^3$) / Post-Bake Out ($\mu g/m^3$) 1st Floor 51 / 65 2nd Floor 32 / 38 Plenum 34 / 38 Outdoor BD / BD
Grot, et al. (1991)	USA	Office (1)	230 ft^2/Person	Not Given	Sodium bisulfite impregnated filter	Formaldehyde 0.02-0.06 ppm
Hedge, et al. (1990)	USA	Office Buildings (2)	Not Given	Not Given	EPA Method 10-11 3 hr samples	Mean Formaldehyde (ppm) AM / PM SP 0.023 / 0.019 SP=Smoking Prohibited SR (Office) 0.008 / 0.012 SR=Smoking Restricted SR (Smoking) 0.018 / 0.023
Hollowell and Miksch (1981)	USA	Home (1) Office (1)	Not Given	0.4 ach	Not Given	Formaldehyde in new home Unoccupied, no furniture 80± 9% $\mu g/m^3$ Unoccupied, with furniture 223± 7% $\mu g/m^3$ Occupied, day 261±10% $\mu g/m^3$ Occupied, night 140±31% $\mu g/m^3$ Outside <20 Aldehydes in an office building ($\mu g/m^3$) Formaldehyde 49 Formaldehyde from various indoor environments Location / Range (ppm) / Mean (ppm) Mobile homes (2), Pa. / 0.1-0.8 / 0.36 Mobile homes w/ complaints (Wash.) / 0-1.77 / 0.1-0.44 Mobile homes w/ complaints (Minn.) / 0-3.0 / 0.4 Mobile homes w/ complaints (Wisc.) / 0.023-4.2 / 0.88
Lofroth, et al. (1989)	USA	Tavern	5-25 people 180 m^3	Not Given	Pump w/sorbent 3 & 4 hr samples HPLC Analysis	1st Study 3 Hr / 2nd Study 4 Hr ($\mu g/m^3$) Formaldehyde 104 / 89

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Table 5. Continued.

Proskiw, et al. (1989)	Canada	Homes (20)	Not Given	see results	Passive dosimeter One week

House type	Formaldehyde (ppm) N	Mean	Median	Vent. rate (ach) N	Mean	Median
R-2000						
Mar 86	6	0.061	0.060	6	0.28	0.26
Aug 86	16	0.058	0.057	-	----	----
Oct 86	16	0.080	0.072	16	0.30	0.29
Feb 87	16	0.064	0.061	16	0.43	0.45
Total	54	0.064	0.061	37	0.35	0.36
Conventional						
Mar 86	4	0.078	0.077	4	0.16	0.44
Aug 86	4	0.067	0.065	-	----	----
Oct 86	3	0.090	0.086	4	0.15	0.17
Feb 87	4	0.067	0.060	4	0.20	0.18
Total	15	0.074	0.066	12	0.17	0.17
Smoking homes		0.065	0.063			
Nonsmoking homes		0.068	0.063			

Quackenboss, et al. (1989b)	USA	Homes (151)	Not Given	Not Given	Passive Samplers Sodium bisulfite One week, 3 sites/house

Building type	Evap. Cooler	Mean $\mu g/m^3$	N
Single fam.	Yes	27	50
	No	37	52
	Total	32	102
Mobile/trailer	Yes	36	6
	No	68	10
	Total	49	10
Apt./Condo.	Yes	25	5
	No	51	24
	Total	47	29
All types	Yes	27	62
	No	43	83

Sterling, et al. (1988)	USA & Canada	Offices (259)	Not Given	Not Given	Not Given

Formaldehyde ND - 1.9 ppm; 0.01, median

Stock (1987)	USA	Homes (41)	Not Given	Not Given	Passive badges one week samples two samples/home

Area	Formaldehyde (ppm) N	Mean	Range
Clear Lake	27	0.07	0.03-0.15
Sunnyside	14	0.10	0.06-0.18

Smoking (cig/day)	N	Mean
<1	26	0.07
1-4	4	0.13
5-10	6	0.07
$\geq$ 20	7	0.07

Yocom (1982)	USA	Homes (2)	Not Given	Not Given	Not Given

House	Formaldehyde ($\mu g/m^3$) Range	Average	Outdoor
ISUERH	34-75	42	<9
ERHH	54-182	120	<12

Yuill & Comeau (1989)	Canada	Homes (50)	Not Given	0.29, Mean ach 0.00-1.35	Passive bubbler colorimetric 2 hour samples

Room	Formaldehyde (ppm) N	Ave.	Max.	Min.
Living room	50	0.090	0.261	0.000
Basement	45	0.088	0.235	0.000
Bedroom	49	0.092	0.285	0.000

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Table 6. Benzene measured under realistic conditions (ppb = 3.2 μg/m³).

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Bayer & Black (1987)	USA	Offices	Not Given	Mechanical	Personal Pump w/Tenax, 4 hr samples(3S,3NS)	Smoking / Non Smoking Benzene 2.0ppb / 1.2ppb
Chan, et al. (1990)	Canada	Homes (12)	Not Given	Not Given	Tenax/Charcoal 2 outdoor, 4 indoor samples each, 90 min. samples, 5-50 liters/ sample	Frequency, Range, and Avg. (μg/m³) Outdoors/Indoors 12 Homes Ambient Air (Nov/Dec): F / Range / Avg. — Indoor Air: F / Range / Avg. Benzene 12 / 1-11 / 7.3 — 12 / 5-59 / 14.8 Frequency, Range, and Avg. (μg/m³) Outdoor/Indoors 6 Homes Ambient Air (Feb/Mar): F / Range / Avg. — Indoor Air: F / Range / Avg. Benzene 6 / 2-8 / 6.0 — 6 / 7-15 / 10.6
Girman, et al. (1989)	USA	Office Building (52,000 ft²)	Not Given	Mechanical	Pump w/Tenax Sorbent, GC/MS	Concentrations (μg/m³) Pre-Bake-Out: 1st Fl. / 2nd Fl. / Plenum / Roof — Post-Bake-Out: 1st Fl. / 2nd Fl. / Plenum / Roof Benzene 2.8 / 3 / 3 / 1 — 0.8 / 1.5 / BD / BD (BD = Below Detection)
Lewis (1991)	USA	Homes (10)	Not Given	.2-.8 ACH	SS Canister 12 hr samples, GC/MS	Avg. Indoor Conc. from OUTdoor Sources and Indoor Souces (ppbC) Compound: R05 / R08 / R11 / R14 / R17 / R20 / R26 / R29 / ALL Benzene OUT 11 / 17 / 20 / 11 / 14 / 15 / 30 / 13 / 15 IN 2 / -1 / 2 / 1 / 0 / 1 / -1 / 2 / 1
Lofroth, et al. (1989)	USA	Tavern	5 - 25 people 180 m³	Not Given	Stainless steel canister, GC/MS	First Study (3hr): Indoor / Outdoor — Second Study (4hr): Indoor / Outdoor (μg/m³) Benzene 27 / 6 — 21 / 8
Pleil, et al. (1986)	USA	Homes (26)	Not Given	Not Given	Summa Cannisters GC/FID,ECD	Indoor Air (PPBV): Summer Data 15 Samples — Winter Data 16 Samples; Outdoor Air: Summer & Winter 6 Samples Each # Obs. / Mean / Range — # Obs. / Mean / Range — # Obs. / Mean / Range Benzene 15 / 7.6 / 0.39-48 — 16 / 4.8 / 0.53-23.6 — 12 / 0.57 / 0.33-0.77
Proctor (1989)	UK	Offices (10) Trains (20)	Not Given	Not Given	Pump w/Tenax Sorbent, 1 hr. samples,	A. Mean / Median / Range — A. Mean / Median / Range (μg/m³) Benzene 6 / 3.1 / 0.6 - 26 — 11.8 / 11.6 / 0.9 - 28.6 Offices(NS) — Train(NS) Benzene 12 / 10 / 3 - 31 — 7.4 / 5.1 / 2.9 - 29.3

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Table 6. Continued.

Proctor, et al. (1989a)	UK	Betting Shops (6)	See chart	Not Given	Tenax TA, 3 l/hr. briefcase, GC/MS	

Compound	N	Site/smoke	Pop/smokers	Qty. Mean ($\mu g/m^3$)
Benzene	3	A/S	20/5	14.3
	2	B/S	10/2	14
	2	C/S	7/3	21
	2	D/S	15/5	18.5
	2	E/S	10/2	18.5
	1	E/Outside	Hvy. Traffic	22
	2	F/NS	11/0	9.5
	2	F/Outside	Med. Traffic	15

Proctor, et al. (1989b)	UK	Office (1)	265 ft^2/person	Not Given	Tenax, 10 ml/min. GC/MS, pump Concs in $\mu g/m^3$	

Compound	Smokers' Office A. Mean	Smokers' Office Median	Nonsmokers' Office A. Mean	Nonsmokers' Office Median
Benzene	13	8	12	10

Proctor, et al. (1991)	UK	Homes & work	Varied	Varied	Personal sampler 24 hour samples, 52 women	

Compound	Smoke/Work Mean	Smoke/Work Median	Smoke/Work Range	Smoke/No work Mean	Smoke/No work Median	Smoke/No work Range	No smoke/Work Mean	No smoke/Work Median	No smoke/Work Range	No smoke/No work Mean	No smoke/No work Median	No smoke/No work Range	All Subjects Mean	All Subjects Median	All Subjects Range
Benzene	15.7	13.3	3.2-48.7	21.6	13.4	5.2-103	60.7	15.5	0.7-510	13.2	10.4	0.2-32.1	26.5	12.8	0.2-510

Sheldon, et al. (1988b)	USA	Hospital (1) Nursing Home (2) Offices (3)	Not Given	Not Given	Pump w/Tenax 12 hr sample GC/MS	

Mean Concentration (ng/L)

	Martinsburg, WV Hospital (new)[a] Trip 1 (7/84)	Trip 2 (10/84)	Trip 3 (8/85)	Fairfax, VA Office (new)[b] Trip 1 (1/85)	Trip 2 (4/85)	Worcester, MA Nursing Home (new)[c] Trip 1 (4/85)	Trip 2 (8/85)	Washington, DC Office (old) Trip 1 (8/84)	Cambridge, MA Office/School (old) Trip 1 (2/85)	Martinsburg, WV Nursing Home (old) Trip 1 (7/84)
Benzene	1.55	2.13	2.88	2.74	4.95	1.70	2.44	5.61	4.50	3.13

[a]Building completed approximately 34 weeks before first monitoring trip.
[b]Building completed approximately 1 week before first monitoring trip.
[c]Building completed approximately 4 weeks before first monitoring trip.

Sheldon, et al. (1988a)	USA	Office Building (1)	Not Given	Not Given	Pump w/Tenax 12 hr sample GC/MS	

Concentration ($\mu g/m^3$)

	Indoors[a] July	Sept.	Dec.	Outdoor[b] all trips
Benzene	5	7	7	3

([a]Mean of six 12-hr avgs. at 5 indoor locations. [b]Mean of 18 12-hr avgs. at one outdoor location.)

Sheldon, et al. (1990)	USA	Homes (12)	16 individuals	Not Given	24 Hour Samples w/Tenax, GC/MS	

Mean Concentration (ng/l) ± S.D.

	Outdoor (n=4)	Indoor (n=12)	Personal (n=8)
Benzene	12±8.8	8.7±2.6	11±4.0

Sterling, et al. (1988)	USA & Canada	Offices (see table for numbers)	Not Given	Not Given	Not Given	

Compound	N	Range	Median
Benzene	27	ND-1.4 mg/m^3	trace

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Table 6. Continued.

Wallace, (1986)	USA	Various (TEAM study)	Not Given	Not Given	Personal and Outdoor samples	Population-Weighted Arithmetic Mean Exposures (24 Hour Samples) $\mu g/m^3$

Sites	Benzene PE[a]	Benzene Out
New Jersey		
Fall 1981	28	9
Summer 1982	NC[b]	NC
Winter 1983	NC	NC
N. Carolina	9	12
N. Dakota	NC	NC
California		
LA:Winter84	18	16
LA:Spring84	9	4
CC:Spring84	7	2

[a]Personal Exposure

[b]Not Calculated--did not meet quality control criteria.

Wallace, et al. (1987)	USA	Homes	Not Given	Not Given	Personal Pumps w/ Tenax sorbent, 12 hr samples GC/MS	Personal Air Exposures-Unweighted Geometric Mean

	n S	n NS	Benzene S	Benzene NS
NJ(F)	153	188	18	11
NJ(S)	69	75	NR	NR
NJ(W)	24	22	NR	NR
LA(W)	29	85	18	14
LA(SP)	11	40	8	7
AP(SP)	19	49	8	7
Overnight Indoor Conc.-Weighted Geometric Mean ($\mu g/m^3$)				
NJ(F)	252	94	16	8.4
NJ(S)	111	44	NR	NR
NJ(W)	37	12	NR	NR
LA(W)	56	58	17	11
LA(SP)	23	28	4.8	4.5
AP(SP)	35	33	4.9	4.4

F=Fall S=Summer W=Winter SP=Spring

Weschler, et al. (1990)	USA	Office Building	Not Given	Not Given	Passive Samplers 4 Periods During 1 Year	VOC ($\mu g/m^3$) Building 2 on 4th Floor During Four Sampling Periods

	6/25/87-7/16/87	9/9/87-10/1/87	11/6/87-12/4/87	3/3/88-3/15/88
Benzene	ND	ND	1.3	ND

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Table 7. Nitrosamines measured under realistic conditions.

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Hoffman, et al. (1987)	USA & Austria	Various	Not Given	Not Given	Pump w/liquid traps, GC/TEA, 1-4 hr continuous samples	

	NDMA	NDEA ($\mu g/m^3$)
Train-Bar	0.11-0.13	ND
Bar	0.24	ND
Sports Hall	0.09	ND
Betting Parlor	0.05	ND
Residence, NS	<.003	ND
Office	0.03	0.03
Conference Room	0.02-.033	0-.02
Work Room	0.023	0
Restaurant	0-0.05	0
Dance Hall	0.07	0.2

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Stehlik, et al. (1982)	Austria	Offices Restaurants Bars	see chart	Not Given	Pump w/liquid traps, GC/TEA, 1-4 hr continuous samples	

Room size	Room Type	# of People	Tobacco Consumed	NDMA (ng/L)	NDEA (ng/L)
$207 m^3$	Working Room	7	Continuous (2hr)	0.024	BD
301	Conference Room	15	26 cig., 1 pipe 6 cigarillos (2hr)	0.031	BD
70	Office	6	27 cig. (2hr)	0.03	0.03
50	Sm. Conf. Room	12	37 cigs., 4 pipes 3 cigars (2hr)	0.02	0.02
120	Suburban Rest.	20	20-30 cigs, 2 pipes (2hr)	BD	BD
160	Vienna Rest.	23	20 cigs., (1hr)	0.01	BD
180	Vienna Rest. 2	25	25-30 cigs., (1hr)	0.04	BD
160	Vienna Rest. 3	23	15-20 cigs., (1hr)	0.05	BD
320	Dancing Bar	30-70	Not Determ.(4hr)	0.07	0.2

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Table 8. PHA measured under realistic conditions.

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
U.S. EPA (1988)	USA	Homes (8)	Not Given	Not Given	Pump w/XAD-4 Sorbent, GC/MS 8 hr Samples	HOME (ng/m^3) (Selected PAH measured in living rooms)

Compound	1NS	2S	3S	4S	5S	6S	7NS	8NS	A. Mean S	A. Mean NS
Naphthalene	800	1100	1100	1100	2200	4200	1800	880	1940	1160
Acenaphthylene	18	37	17	48	120	59	10	8.9	56	12
Phenanthrene	71	87	80	200	210	110	59	86	137	72
Fluoranthene	7.6	8.6	6.5	19	23	6.4	7.2	12	13	9
Pyrene	4.6	5.2	4.0	11	17	9.5	5.6	7.4	9	6
Benzo(e)pyrene	0.74	3.2	1.3	3.7	10	6.5	0.68	1.4	5	1
Benzo(a)pyrene	0.28	1.2	0.34	1.4	3.3	1.4	0.31	0.83	1.5	0.47
Quinoline	9.4	240	76	480	1100	300	23	9.1	439	15.[illegible]
Isoquinoline	21	140	34	300	620	170	35	16	25[illegible]	[illegible]

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Daisey, et al. (1989)	USA	Homes (7)	Not Given	0.13:0.89 ACH	Pump w/Teflon coated fiberglass filter, 48 hr samples	PAH (ng/m^3)

House	Wood burning	FLU	PYR	BEP	BBF	BKF	BAP	BGHIP	[illegible]	TOTAL
102	+	0.72	0.47	0.48	0.52	0.24	0.[illegible]	1.25	[illegible]	6.1
	-	1.18	0.98	0.41	0.24	0.10	0.17	0.15	[illegible]	3.4
106	+	0.32	0.62	0.65	0.68	0.29	1.24	6.20	3.5	13.5
	-	0.58	0.70	0.42	0.11	0.04	0.11	0.33	0.[illegible]	2.4
108	+	0.96	0.50	0.38	0.40	0.19	0.43	0.55	[illegible]	4.5
	-	0.11	0.03	<0.06	0.04	0.02	0.03	0.08	0.[illegible]	0.4
203	+	0.81	0.58	0.95	0.51	0.21	0.40	0.75	0.[illegible]	5.2
	-	0.07	0.14	<0.06	<0.007	0.007	<0.009	<0.01	<0.[illegible]	0.3
204	+	0.16	0.02	0.14	0.10	0.03	0.07	0.12	0.[illegible]	0.8
	-	0.09	0.22	0.07	0.02	0.01	<0.009	0.02	0.0[illegible]	0.5
208	+	0.65	0.65	1.36	1.22	0.48	1.34	0.87	[illegible]	8.8
	-	0.07	0.05	<0.06	0.009	0.005	<0.009	<0.01	<0.[illegible]	0.2
300	+	0.18	1.53	0.31	0.27	0.09	0.33	0.25	0.3[illegible]	3.4
	-	0.50	0.31	0.43	0.04	0.02	0.02	0.10	0.1[illegible]	1.6

(FLU=Fluoranthene, PYR=Pyrene, BEP=Benzo(e)pyrene, BBF=Benzo(b)fluoranthene, BKF=Benzo(k)fluoranthene, BAP=Benzo(a)pyrene, BGHIP=Benzo(ghi)perylene, IND=Indeno[illegible],2,3,-cd)pyrene)

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Grimsrud, et al. (1990)	USA	Office Buildings (40)	Not Given	Not Given	Gravimetric PM3.0 126 RSP samples analysed for B(a)P	

	Mean Concentration B(a)P ([illegible])
Non smoking Areas	0.4
Smoking Areas	1.1

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Lioy, et al. (1987)	USA	Homes (4)	Not Given	Not Given	PM10 extraction	

Home	N	B(a)P G. Mean	Maximum (ng/m^3)
1	14	0.5	4.8
2	13	1.1	4.4
3	14	0.8	2.3
6	13	0.3	1.4

Author & Date	Country	Building Type	Occupancy	Ventilation	Sampling	Concentrations/Comments
Waldman, et al. (1989)	USA	Homes	Not Given	Not Given	PM10 extraction	BaP Range nd : 8.6 ng/m^3

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receive would provide an independent assessment as to whether these health claims are realistic.

[illegible] Dose is defined as the amo[illegible]

[illegible]

[illegible] dose is a function of the concentration of [illegible] substance, the duration of exposure, the rate [illegible] respiration, and the percentage of material potential[illegible] [illegible] retained by the lungs.

Concentration: Concentrations of RSPs were determined as the mean difference between nonsmoking and smoking conditions as shown in Table 9.

Duration of exposure: In order to determine the duration of exposure in each of the five categories, data on time activity patterns were needed. Though there were several references available describing studies which performed time/activity surveys of one type or another, there were few that were adequate. The requirements that needed to be met included:

1. Data needed to be American or Canadian
2. Study needed to be of adults
3. Study could not be of select subpopulations
4. Study needed to include most of the entire day.

Five publications met the four chosen criteria (Jenkins et al. 1990; Juster and Stafford 1990; Shaw 1983; Spengler et al. 1985; Szalai et al. 1972). Each author's activity categories were allocated to one of the following locations that were chosen to match exposure categories used for the literature review.

```
Location                 Exposure Category

[illegible] ------------ Home
[illegible] Work ------------------- Office [illegible]
[illegible] Public Transportation----- Public transportation
[illegible] Restaurant --------------- Restaurant
6.  Bar ---------------------- Bar/Taverns
7.  Other, public ------------ Office, workplaces
                      (similar to office environment)
8.  Outdoor ------------------ None
```

Time allocations for each category were then averaged to produce an average day based on the five studies reviewed. Time spent in each category is expressed separately for males, females, employed, and unemployed persons. Time spent in each activity category is expressed in minutes per day and hours per day averaged over seven days per week. If the average day did not add up to 1440 min (24 h), each category was adjusted by the percentage of the error to produce a 24-h day.

There were fewer data available regarding employed/unemployed time activity patterns than for male/female time activity patterns. Because of this, it was necessary to subdivide some authors' categories into more than one of the five chosen for

Table 9: Mean respirable particles $\mu g/m^3$ in smoking and nonsmoking areas in real-life situations.

		Smoking			Nonsmoking			Diff. in Means:
Category	# of Studies	N	Mean	Range	N	Mean	Range	S - NS
Homes	14	951	49.5	17-212	905	22.3	7-77.[illegible]	27.2
Offices and Public Places	24	805	67.7	12-2700	640	45.9	nd-240	21.8
Restaurants	11	257	131.5	nd-685	335[b]	89.7	c	41.8
Bars/Taverns	4	9	103.7	c	c	c	c	103.7[d]
Trains	1	20	216	70.8-325	20	185	63.3-450	30

a - The mean is calculated by weighting the mean values in each study based on sample size and the calculating an overall arithmetic mean. If actual values for nonsmoking or smoking areas were not given, but UVPM values were available, the UVPM values were utilized in obtaining the weighted means. For example, if a total RSP mean value and UVPM mean value were available for a smoking area but not for a nonsmoking area, a nonsmoking area value was obtained by subtracting the UVPM value from the total RSP value.

b - Derived from total RSP-UVPM.

c - No ranges available; no data available.

d - Some of this RSP would be due to sources other than tobacco smoke. If properties are similar to restaurants, this mean would be approximately 48.3.

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Table 10. Carbon monoxide concentrations (ppm) in smoking and nonsmoking areas in real-life situations. Conversion factor: 1ppm = 1.15 mg/m^3.

Category	No. of Studies	Smoking N	Smoking Mean	Smoking Range	Nonsmoking N	Nonsmoking Mean	Nonsmoking Range	Diff in Means S - NS
Offices and Public Bldgs.	10	597	2.55	0.1-8.7	275	2.99	0.7-4.0	-0.04
Restaurants	5	107	3.5	0.4-9.0	---	---	---	---
Taverns/Bars	2	5	6.4	-------	---	---	---	---
Trains	2	18	2.2	1.0-5.2	10	1.30	0.5-2.9	0.90
Buses	1	35	6.0	3.7-10.2	---	----	-------	---
Autos	1	---	----	--------	213	11.6	8.8-22.3	---

Homes - Very little information on homes
Yuill et al. (1989); 76 homes, 0.7 mean, range 0-4.0
No data on smoking or nonsmoking.

Table 11. Nicotine concentrations ($\mu g/m^3$) in smoking and nonsmoking areas in real-life situations.

Category	Smoking No. Studies	Smoking Sample Sites	Smoking Mean	Smoking Range	Nonsmoking No. Studies	Nonsmoking Sample Sites	Nonsmoking Mean	Nonsmoking Range
Offices and Public Bldgs.	14	673	6.2	ND-69.7	5	270	0.3	0.1-2.1
Restaurants	10	390	5.7	0-37.2				
Taverns/Bars Betting Shops	4	17	19.1	3-65.5	1	2	1.2	0.4-2.0
Homes	11	98	3.7	0.1-12.0	3	28	0.29	0-1.0
Trains	1	20	15.3	0.6-49.3	1	20	4.5	0.5-21.2

this review. To do this, the proportion determined from the male/female time activity pattern was used to divide that author's category. For example, time at home for employed and unemployed persons was determined by Spengler et al. (1985) but not divided into waking and sleeping time. The amount of time determined to be spent sleeping in the male/female time allocation was subtracted from Spengler's home time to produce home-awake and home-asleep values for employed/unemployed persons. The time activity patterns calculated from the literature are listed in Tables 12 and 13.

Respiration rates: Respiration rates were taken from Rosenblatt et al. (1982):

1) Adult male, light work—28.6 L/min (1.7 m^3/h)
2) Adult male, resting—7.43 L/min (0.4 m^3/h)
3) Adult female, light work—16.3 L/min (1.0 m^3/h)
4) Adult female, resting—4.5 L/min (0.3 m^3/h).

These figures assume 16 h of rest and 8 h of light work per day, and are in agreement with those from other sources (USEPA 1989).

An average of approximately eight hours per day is spent sleeping. This leaves eight waking hours per day spent at "light work" and eight hours spent "resting". Since no single activity period is likely to be all "light work" or all "resting", the two values were averaged to produce a respiration rate for "awake". These values are as follows:

Male, awake—1.05 m^3/h
Male, asleep—0.4 m^3/h
Female, awake—0.65 m^3/h
Female, asleep—0.3 m^3/h.

Retention efficiency: Retention efficiency for sidestream smoke particles has been reported by Hiller et al. (1982, 1987) to be 11%.

Results of dosimetric calculations

In any exposure situation, the retained dose may be calculated by the following equation:

Dose = Concentration × duration × respiration rate × % retention.

[illegible]

[illegible] is taken from the time/ac- [illegible]

Respiration rates are calculated from Rosenblatt. The "home, asleep" category is given the resting respiration rate. All other categories are assigned the "awake" respiration rate.

Percent retention is 11% as calculated by Hiller.

Exposure to ETS can come in any number or combination of situations. To estimate the breadth of possible ETS exposures, doses of ETS have been calculated for four different scenarios:

1) Male and female—exposed at home, in restaurants, bars and other public places, but not at work or while traveling. This essentially calculates the ETS exposure one may expect during nonworking hours.

2) Male and female—exposed only at work and while traveling (assume all transportation by train). This will produce a conservative estimate of occupational exposures.

3) Worst case exposure, male and female—exposed at home and while traveling, works in a bar and is also exposed in other public places (assumes use of the average exposure values).

4) Employed vs. unemployed males—exposed in all facets of life, employed person works in office environment. Employed and unemployed females would be essentially the same but with different respiration rates.

The calculations for each are as follows:

[illegible] taurants, bars and other public places, but not at work or while traveling. Dose = (concentration; home × duration; awake × respiration rate; awake × retention) + (concentration; home × duration; asleep × respiration rate; asleep × retention) + (concentration; restaurant × duration x respiration rate x retention) + (concentration; bar × duration × respiration rate × retention) + (concentration; offices, × duration; other, public × respiration rate × retention):

Male Dose = $(27.2 \times 6.4 \times 1.05 \times 0.11) + (27.2 \times 7.5 \times 0.4 \times 0.11) + (41.8 \times 0.5 \times 1.05 \times 0.11) + (103.7 \times 0.8 \times 1.05 \times 0.11) + (21.8 \times 1.6 \times 1.05 \times 0.11)$

Male Dose = 45.11 µg/day, 16.46 mg/y

Female dose = $(27.2 \times 8.7 \times 0.65 \times 0.11) + (27.2 \times 7.8 \times 0.3 \times 0.11) + (41.8 \times 0.4 \times 0.65 \times 0.11) + (103.7 \times 0.9 \times 0.65 \times 0.11) + (21.8 \times 1.6 \times 0.65 \times 0.11)$

Female Dose = 34.28 µg/day, 12.51 mg/y

2) Male and female—exposed only at work and while traveling.

Male Dose = 17.19 µg/d, 6.27 mg/y

Female dose = 6.94 µg/d, 2.53 mg/y

3) Maximum exposure, male and female—exposed at home and while traveling, works in a bar and is also exposed in other public places.

Male Dose = 108.65 µg/d, 39.66 mg/y

Female Dose = 57.62 µg/d, 21.03 mg/y

Table 12. Total time allocations for male/female.

Location	Males		Females	
	Min/day	Hr/day	Min/day	Hr/day
1. Home, awake	386	6.4	521	8.7
2. Home, Asleep	452	7.5	470	7.8
3. Work	296	4.9	167	2.8
4. Travel	81	1.4	67	1.2
5. Restaurant	31	0.5	26	0.4
6. Bar	48	0.8	54	0.9
7. Other, public	94	1.6	95	1.6
8. Outdoor	52	0.9	40	0.7
TOTAL	1,440	24.0	1,440	24.1*

* Totals do not add up to 24 hours due to rounding.

Table 13. Total time allocations, employed, and unemployed persons.

Location	Employed		Unemployed	
	Min/day	Hr/day	Min/day	Hr/day
1. Home, Awake	427	7.1	733	12.2
2. Home, Asleep	511	8.5	499	8.3
3. Work	276	4.6	5	0.1
4. Travel, Public	81	1.4	37	0.7
5. Restaurant	17	0.3	20	0.3
6. Bar	33	0.6	36	0.6
7. Other, Public	61	1.0	68	1.1
8. Outdoor	34	0.6	42	0.7
TOTAL	1,440	24.1*	1,440	24.0

* Totals do not add up to 24 hours due to rounding.

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4) Employed vs. unemployed males—exposed in all facets of lifestyle, employed person works in office environment.
Employed Male Dose = 60.06 μg/d, 21.92 mg/y
Unemployed Male Dose = 62.34 μg/d, 22.76 mg/y.

There are several chemical and exposure pattern differences between direct smoking and ETS exposure. However, expressing ETS exposure in cigarette equivalents is one way to develop a sense of the magnitude of exposure.

Arundel et al. (1988) has calculated a retained dose of particles of 10.56 mg/cigarette and 8.48/mg cigarette for male and female direct smokers, respectively. Using these figures, the dose in cigarette equivalents of particles for each of the four exposure scenarios becomes:

1) Exposed at home, in restaurants and in bars:
Male—1.56 cigarette equivalents per year
Female—1.48 cigarette equivalents per year.

2) Exposed at work and travel only:
Male—0.59 cigarette equivalents per year
Female — 0.30 cigarette equivalents per year.

3) Maximum, works in a bar, exposed in all facets of life:
Male—3.76 cigarette equivalents per year
Female—2.48 cigarette equivalents per year.

4) Employed vs. unemployed males:
Employed—2.08 cigarette equivalents per year
Unemployed—2.15 cigarette equivalents per year.

Clearly, such calculations of cigarette equivalents have little or no relevance to any assessment of the potential risk from exposure to ETS. The mainstream smoke particles inhaled during the act of puffing on a cigarette will be quite different from ETS particles in terms of their precise chemical composition, their size distribution and the route in which they are taken into the body. However, the calculation of cigarette equivalents does at least allow a subjective impression of the relative doses involved in active smoking compared to everyday exposure to ETS.

DISCUSSION

Indoor air and ETS review

Respirable particulate matter: Table 9 shows the mean RSPs in smoking and nonsmoking areas in real life conditions. The mean differences between nonsmoking and smoking conditions are 27.2 $\mu g/m^3$ for homes, 21.8 for offices and public facilities, 41.8 for restaurants, in excess of 48.3 for bars and taverns, and 30 for trains.

Carbon monoxide: Table 10 shows carbon monoxide concentrations (ppm) in smoking and nonsmoking areas in real life conditions. In offices, where the data allowed for a smoking/nonsmoking comparison, the mean difference was 0.28 μL/L (0.32 mg/m^3). Because of the magnitude of this difference compared to the mean levels measured and ranges reported, the significance of this difference must be questioned. There are essentially no data since 1982 on nonsmoking conditions in restaurants or bars. The slightly higher levels in these areas indicate that smoking may account for at least part of the CO.

There is little information on CO in homes. Yuill and Comeau (1989) report on home CO, but they do not indicate whether smoking was present.

The information on CO levels in transportation vehicles indicates little difference between smoking and nonsmoking areas on trains. Buses have higher levels (6.0 μL/L; 6.9 mg/m^3) of CO; but from the information presented it is impossible to determine if the concentration is affected by tobacco smoke or by vehicle exhausts. The higher level of CO (11.6 μL/L; 13.3 mg/m^3) in autos where no smoking took place indicates that the impact is from vehicle exhausts (Flachsbart et al. 1987).

Nicotine: Table 11 shows nicotine concentrations in smoking and nonsmoking areas. Nicotine, as expected, is considerably lower in concentration in nonsmoking compared to smoking areas. The means of 6.2, 5.7 and 3.7 $\mu g/m^3$ for offices and public buildings, restaurants and homes, respectively, where smoking occurred, are extremely low exposures compared to the levels of many other volatile and semivolatile substances found in indoor air. Smoking sections of trains are slightly higher and, as expected, bars and taverns exhibited the highest mean levels (19.1 $\mu g/m^3$).

Nitrogen dioxide: Most studies which measure nitrogen dioxide (NO_2) in the indoor environment evaluate the effect various appliances, such as gas stoves or kerosene heaters, have on NO_2 concentrations. Only three studies attempted to evaluate the impact of ETS on NO_2 levels in homes (Dumont 1986; Good et al. 1982; Hosein et al. 1985). Houses where smoking occurred had approximately 3.0 $\mu g/m^3$ higher nitrogen dioxide levels than houses with no smoking. There are no studies which evaluate the effect of ETS on NO_2 levels in offices, restaurants, bars, or transport vehicles.

Formaldehyde: There is a substantial amount of information regarding formaldehyde levels indoors. However, little of it evaluates the potential effect of [illegible] levels. Of the literature reviewed, [illegible] homes (Stock 1987) and one study of two office buildings (Hedge et al. 1990) compared formaldehyde levels to smoking status. The residential study found no increase in formaldehyde levels with increased levels of smoking. The office study found approximately a 10 nL/L (12.3 $\mu g/m^3$) increase in formaldehyde levels in a designated smoking area over a nonsmoking area of one building. However, there was no difference between the designated smoking area in one building and another nonsmoking building. Because of the limited number of samples and the number of confounding sources for formaldehyde these results must be interpreted with caution.

Benzene: Five studies contained benzene data in smoking and nonsmoking areas (see Table 6). In two of these studies, nonsmoking areas reportedly had higher concentrations of benzene. In three studies, nonsmoking areas had lower concentrations than smoking areas. The differences are minimal, especially when outdoor concentrations also are considered.

The assumption was made that in studies where smoking/nonsmoking was not reported by the author, that there was some smoking occurring. Where smoking is assumed to take place, the highest mean level recorded was 15.7 $\mu g/m^3$. However, in nonsmoking areas the highest mean was 60.7. In summary, there are few data to support a conclusion that smoking has a significant impact on benzene concentration in offices.

The data on benzene in homes suggest that smoking homes have a higher concentration than nonsmoking homes by approximately 3.5 $\mu g/m^3$. It is difficult to separate home-only data from personal exposure monitor data except for two studies where indoor air benzene ranges from 4.8-14.8 $\mu g/m^3$. The one overnight study in homes by Wallace et al. (1987) shows an increase of approximately 3.1 $\mu g/m^3$ in smoking homes vs. nonsmoking homes.

There are few data on benzene on public transportation. A paper by Proctor (1989) on 20 trains shows a slightly higher mean concentration of benzene in smoking versus nonsmoking areas. The mean differences are small.

Polyaromatic hydrocarbons (PAH's) and nitrosamines: PAH's are commonly found in indoor air. Except for naphthalene, quinoline, and isoquinoline, the levels measured are in the low ng/m^3 range (Table 8). Few studies have compared PAH levels in smoking and nonsmoking environments. The [illegible] review which [illegible] areas indicate that between 50% and [illegible]% of the concentration of various PAHs may come from [illegible] smoke. Because of the paucity of data [illegible] interpreted with caution. The presence of these substances has also been documented in wood smoke, automotive exhaust, foods, alcoholic beverages, and cosmetics (NTP 1989).

Two studies have reported the presence of N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) in smoke-filled rooms (Table 7). These are not tobacco-specific nitrosamines. The lack of reported background levels and the unusually high level of smoking prevents the evaluation of ETS contribution to these substances. Other nitrosamines reported to be found in tobacco smoke have either not been monitored or not been reported in ambient air where ETS is present.

The U.S. Surgeon General (1986) and NRC (1986) reports include summary tables of known or suspect human carcinogens present in concentrated sidestream and mainstream tobacco smoke. Concentrated sidestream and mainstream tobacco smoke are not representative of ambient air ETS because the concentrated smoke is subject to dilution in ambient air, removal by sinks or filtration, and possible transformation (Reasor 1987).

Based on current literature, it appears that ETS has an effect on the levels of nicotine and respirable particles in an indoor environment. There also is a slight increase in NOx levels in the presence of ETS. ETS appears to have less effect on the levels of carbon monoxide, formaldehyde, or benzene.

Odor and irritation: Studies by Cain et al. (1987), Weber (1984), and Weber and Grandjean (1987) indicate that levels of ETS which produce carbon monoxide concentrations below 1.5-2.0 $\mu L/L$ (1.7-2.3 mg/m^3) also will be acceptable to 80% or more of the occupants in terms of irritation, odor, and overall quality. Where a moderate amount of smoking occurs, this level is unlikely to be approached. This conclusion is reflected in ASHRAE Standard 62-1989 (ASHRAE 1989), which does not separate smoking and no-smoking areas in terms of recommended ventilation rates.

It is possible that the 1.5-2.0 $\mu L/L$ (1.7-2.3 mg/m^3) level of carbon monoxide may be exceeded in some cases. This would occur either in heavy smoking situations, such as those found in bars or smoking lounges, or where inadequate ventilation occurs.

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Dosimetric calculations

One can calculate the doses of RSP retained from [illegible] a female exposed only at work to approximately 49 mg/y for a male exposed in all facets of his life. Occupational exposure is only a minor portion of total exposure in most cases. Exposures in one's private life may produce the largest retained dose of ETS particulates.

For exposures received while in transit, it was assumed that all transit time was spent in the smoking section of trains. Actual time allocation data (Jenkins et al. 1990) indicate that only 5% of travel time is spent on public transport. It is believed that when traveling by auto, nonsmokers will generally choose to travel with other nonsmokers. There are no data comparing particulate levels in autos where smoking occurs to levels were there is no smoking. However, carbon monoxide levels of 8.8-22.3 μL/L (10.1-25.6 mg/m^3) found by Flachsbart et al. (1987) suggest that exposures in automobiles come primarily from vehicle exhaust and not ETS. Thus, the choice of trains as a surrogate for ETS exposure while in transit is conservative.

The estimated mean RSP dose of exposure to the population of nonsmoking adults is based on the difference between smoking and nonsmoking environments. There is a possibility that a person could be exposed to the high end of the range of RSPs as reported in the literature. The likelihood is that the bulk of the RSPs in those situations would be from sources other than tobacco smoke.

Persons also may be exposed to the upper limits of ETS—derived RSP ranges. Generally, these are for short time intervals. The mean difference values for the various categories of exposure should reflect the overall minimum and maximum exposure such that the total dose for the year would be included.

Attempts to calculate increased risk or excess mortality from lung cancer (Repace 1985; NRC 1986) and heart disease (Wells 1988; Glantz and Parmley 1991) reportedly resulting from ETS exposure are not uncommon. These calculations, however, rely almost exclusively on epidemiologic studies that have no adequate measure of exposure or dose. Such studies are known to be subject to problems of bias (Lee 1987) and confounding factors (Koo et al. 1988) which have not been taken adequately into account.

One of the paradigms of toxicology is that the magnitude of the dose determines the response. Comparing the dose one may receive from ETS to the magnitude of claimed health effects provides one measure of the accuracy of those claims.

Other studies that have calculated ETS dosage [illegible] discrepancies between the levels of risk calculated [illegible] the epidemiology studies and [illegible] ported by dosimetric calculations. Wells (198[illegible]) [illegible] Repace and Lowrey [illegible] have [illegible] to address this discrepancy. Wells argues that the majority of the "tar" fraction of ETS may be in the vapor phase. He claims that this vapor phase would be retained 100%, producing the majority of the dose one would receive. Thus, he suggests that particles are not an accurate measure of ETS dose. However, the compounds he lists as candidates for vapor phase tar components are not convincing in terms of their potential human health effects. They also are substances that have only been measured in concentrated sidestream and mainstream smoke, not ambient ETS. Until levels of these compounds are actually shown to increase in the presence of ETS, his argument must be considered speculative.

Repace and Lowrey (1991) calculate a daily inhaled dose of ETS particles between 1.4 and 14 mg/d. Calculations based on literature values of ETS concentrations from this paper indicate an inhaled dose of between 0.1 and 0.06 mg/d. When one considers that only 11% of these particulates are retained, the actual dose becomes 0.01 to 0.006 mg/d or 1/140 to 1/233 of the dose claimed by Repace and Lowrey.

Until the problems of confounding and bias in the epidemiology studies are resolved, dosimetric considerations can be the only independent confirmation of the accuracy of their claims. At this point, it can only be concluded that the estimated dose of ETS one can be expected to receive does not support the health risk claims being made by USEPA (1990) and others.

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Uptake of tobacco smoke constituents on exposure to environmental tobacco smoke (ETS)

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Summary. For the purpose of risk evaluation, passive smoking is frequently regarded as low-dose cigarette smoking. However, since the physical, chemical and biological properties of mainstream smoke (MS), which is inhaled by the smoker and environmental tobacco smoke (ETS), which is breathed by the passive smoker are quite different, risk extrapolation from active smoking to passive smoking is of doubtful value. In a series of experimental exposure studies we compared the uptake of tobacco smoke constituents by active and passive smoking. The results show that biomarkers which were found to be elevated after experimental ETS exposure, such as nicotine and cotinine in plasma and urine as well as thioethers in urine, indicate gas-phase exposure in passive smokers, but particle-phase exposure in active smokers. Biomarkers which should indicate the uptake of particle-bound, genotoxic substances with ETS, such as urinary mutagenicity, metabolites of polycyclic aromatic hydrocarbons (PAH) and DNA adducts, were not found to be elevated even after extremely high ETS exposure. From these results we conclude that a risk evaluation for passive smoking on the basis of dosimetric data is currently not possible.

Key words: Environmental tobacco smoke (ETS) – Passive smoking – Smoking – Biomonitoring – Gas phase – Particle phase – Genotoxic substances

Abbreviations: 1-ABP = 4-aminobiphenyl; BaA = benzo(a)anthracene; BaP = benzo(a)pyrene; BE = butanol extraction; BeP = benzo(e)pyrene; CO = carbon monoxide; COHb = carboxyhaemoglobin; DABS = DNA binding substances; DRZ = diagonal radioactive zone; ETS = environmental tobacco smoke; GC = gas chromatography; GC/MS = gas chromatography coupled to mass spectrometry; HPLC = high performance liquid chromatography; HPMA = 3-hydroxypropylmercapturic acid; MS = mainstream smoke; NNK = 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = N-nitrosonornicotine; NO_x = nitrogen oxides (NO/NO_2); PAH = polycyclic aromatic hydrocarbons; PhIP = 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; P1 = nuclease P1; RSP = respirable particles

Chronic exposure of non-smokers to environmental tobacco smoke (ETS), also termed passive or involuntary smoking, has been associated with health damage, in particular lung cancer [26, 70]. The evidence for a causal relationship is rather weak, and primarily based on epidemiological findings and linear risk extrapolations from smokers to passive smokers. Meta-analyses of more than 20 epidemiological studies show a relative lung cancer risk of 1.1–1.3 for non-smokers living with a smoking spouse as compared with those living with a non-smoking spouse [37]. Since such low risks are highly sensitive to bias and confounding factors, which are not eliminated by meta-analysis [15, 69], it is unlikely that the controversy can be solved by epidemiological investigations. Risk extrapolation from active to passive smoking simply assumes that passive smoking is a kind of low-dose cigarette smoking [70]. Doses are expressed as "cigarette equivalents" based on single tobacco-smoke constituents such as nicotine or its major metabolite cotinine [56] or respirable particles [55]. These approaches have been criticized, mainly due to physical, chemical and biological differences between MS and ETS [9].

Table 1 summarizes some major differences between active and passive smoking. In contrast to smokers, passive smokers breath aged tobacco smoke. In vitro tests suggest that aged ETS is less cytotoxic than fresh MS inhaled by the smoker [65]. The smaller size of ETS particles as compared with MS particles [26, 70] and the differences in inhalation patterns between passive and active smoking lead to much lower particle deposition rates of 11% in passive smokers [21] as compared with 50–90% in smokers [22]. Furthermore, the intact clearing mechanism of the respiratory tract of non-smokers removes particles more effectively than the respiratory tract of smokers which may become damaged due to long-term cigarette smok-

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Table 1. Differences between active and passive smoking

Parameter (Reference)	Active smoking	Passive smoking
Age of smoke	Fresh	Aged
Cytotoxicity (65)	High	Low
Particle size (70)	0.2–0.4 µm	0.1–0.2 µm
Inhalation pattern	Intermittent deep bursts	Continuous normal breathing
Particle deposition (21, 22)	50–90%	11–15%
Clearing mechanism (41)	May be damaged	Intact
pH of smoke (33, 70)	6.0–6.2	6.7–7.5
Enzyme induction (46, 54)	High	Negligible

ing [41]. ETS is more alkaline than MS [34], and this results in an increased absorption of nicotine in the oral cavity [28]. Toxifying and detoxifying enzymes have been found to be induced in smokers [46, 54]; Whether this is beneficial or harmful for the exposed individual is not known. Finally, risk extrapolation to very low doses is extremely uncertain since the shape of the dose–response curve in this range is based on assumptions and not on experimental or epidemiological data [12].

Estimates for the uptake of gas-phase and particulate-phase constituents by active and passive smoking are presented in Table 2 based on the different deposition rates for smoking and ETS exposure [21, 22]. The theoretical uptake of particle-bound substances is 2–3 orders of magnitude higher in smokers than in passive smokers, whereas the ratio is about 5 for gas-phase constituents under extreme exposure to ETS. This could be of importance with respect to lung cancer risk by passive smoking, considering the fact that the tumorigenic effect of tobacco smoke is mainly attributed to the particle phase [26].

In a series of experimental studies, we quantified the uptake of tobacco smoke constituents by active and passive smoking in order to verify these theoretical considerations. In addition to the most common biomarkers for tobacco smoke exposure such as carboxyhaemoglobin (COHb), nicotine and cotinine in body fluids, we were particularly interested in the uptake of genotoxic substances and their biological effects in the human organism. Furthermore, our efforts were aimed at determining whether or not passive smoking can be regarded simply as low-dose cigarette smoking for risk evaluation.

Methods

Study protocols

Study protocols have been described in detail elsewhere [2, 62–64]. All subjects were healthy male volunteers aged 20 to 40 years. For each study they stayed in the laboratory from the evening of the first experimental day until the morning after the last experimental day. During the study period, smokers were not allowed to smoke except during the special smoking or ETS exposure sessions. These sessions usually last for 8 h and took place in an unventilated, furnished room with five smoking and five non-smoking subjects present. The smokers smoked cigarettes of their usual brand to prescribed schedules. Sham-exposure sessions (control days) were performed in exactly the same way without smoking. During the studies, subjects

Table 2. Estimated uptake doses by active and passive smoking[a]

Tobacco smoke constituents	Smoking (S) (20 cig/d)	Passive smoking (PS) (8 h/d)	Dose ratio S/PS
Gaseous phase			
CO (mg)	40–400	14.4–96	2.7–4.2
Formaldehyde (mg)	0.4–1.8	0.08–0.4	4–5
Volatile nitrosamines (µg)	0.05–1.0	0.03–0.4	1.5–2.5
Benzene (µg)	200–1200	40–400	3–5
Particulate matter			
Particles (mg)	75–300	0.025–0.24	1250–3000
Nicotine (mg)[b]	7.5–30	0.08–0.4	75–90
BaP (µg)	0.15–0.75	0.001–0.011	70–150
Cadmium (µg)	1.5	0.001–0.014	110–1500
Tobacco-specific nitrosamines (µg)	4.5–45	0.002–0.010	2300–4500

[a] Data are compiled according to reference 62

[b] Nicotine is particle-bound in MS and a gas-phase constituents in ETS [14]

ate a defined diet low in PAH [39], which was identical in quality and quantity on each day of the investigations. Urine (24 h) was collected from all subjects beginning at 8 a.m. on each day. Blood was drawn into heparinized tubes immediately before and after each experimental session.

Study 1 [62] comprised four experimental days with eight smokers taking part. Day 1 and day 3 were control days without smoking. On day 2 each subject smoked 24 cigarettes in 8 h (1 cigarette/20 min) through an empty filterholder. On day 4 the same number of cigarettes were smoked with a glass-fibre filter in the holder which removed >99% of the smoke particles. During the smoking sessions, the room was adequately ventilated so that ETS exposure was minimal.

In *Study 2* [62], five smokers and five non-smokers took part over 5 days. On day 1 no smoking, ETS exposure or sham exposure took place. Day 2 and day 4 were control days with 8-h sham-exposure sessions. On day 3 the non-smokers were exposed to the gas phase of ETS for 8 h. For this purpose the non-smokers wore masks equipped with filters (Sekur Polimask-PC, filter classes P1 and P2, Pirelli, Germany), which retained >99% of the respirable particle mass in the inspired air. The smokers who generated the ETS each smoked 24 cigarettes in 8 h (1 cigarette/20 min) and were not exposed to ETS as they smoked cigarettes outside the room through a glass wall with the lit end of the cigarette positioned inside the ETS exposure room. The exhaled smoke was blown through a one-way valve into the exposure room. In order to have the same occupancy, five additional volunteers sat in the experimental room. On day 5 the non-smokers wore no face masks and were thus exposed to whole ETS. The smokers smoked in the experimental room.

Study 3 [63] comprised four experimental days with five smokers and five non-smokers. Day 1 and day 3 were control days. On day 2 and day 4, the non-smokers were exposed to whole ETS generated by the five smokers who each smoked 24 cigarettes during 8 h. On day 4 the smokers smoked through glass-fibre filters and were thus exposed to only the gas phase of MS.

Study 4 [2] had only one experimental day during which eight non-smokers were exposed to the ETS of two smokers for 9 h. Smoking frequency was adusted so that CO concentration fluctuated around 10 ppm.

Study 5 [64] was identical to study 3. However the smokers smoked the whole MS on both exposure days.

Air monitoring

Air sampling tubes were installed at the breathing height of a seated person at the end of the room opposite the seated smokers. CO was measured continuously by an infrared CO monitor (UNOR 6N) (Fa. Maihak, Hamburg, FRG). Nitrogen oxides (NO/NO_2) were detected by chemoluminescence using a Nitrogen-Oxide Analyzer, Model 8840 (Monitor Labs Inc., USA). Nicotine was determined according to the method of Odgen [43]. The alkaloid was absorbed on XAD-4 resin with an air flow rate of 1 l/min. 3-Vinylpyridine was determined in the same samples as nicotine [11]. Sampling times were 4 h on the sham-exposure days and 2 h on the exposure days. Carbonyl compounds (aldehydes) were sampled on Sep-PAK C_{18} (Waters Associates, Milford, Mass., USA) coated with 2,4-dinitrophenylhydrazine and determined by HPLC [35]. Flow rates and sampling times were similar to those for nicotine. Respirable particles (RSP) were determined gravimetrically according to the method of Conner [8]. Solanesol was determined by HPLC in methanol extracts of the filter pads used for particle sampline [45]. The sampling flow rate was 1.5–2.0 l/min. Sampling periods were similar to those for nicotine. PAH were detected according to the method of Grimmer et al. [17]. Benzene was measured by a modification of the NIOSH method [42]. Sampling times ranged from 10 to 60 min with flow rates ranging from 0.5 to 2.0 l/min. The tobacco-specific nitrosamines N-nitrosonornicotine (NNN) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) were determined by GC/thermal energy analyser detection after sample clean-up by column chromatography [33].

Biomonitoring

COHb was determined spectrophotometrically on fresh blood samples with an IL 182 CO-Oximeter (Intrumentation Laboratories Ltd. USA). Nicotine was determined in plasma and urine by gas chromatography [20] and cotinine in plasma and urine by a modified radioimmunoassay method [36]. Thioethers were measured in urine by quantifying the sulphhydryl groups with Ellman's reagent after alkaline hydrolysis [3, 19]. Sulphur-containing acid compounds in urine were profiled by capillary GC with S-specific detection of the methyl esters with

and without silylation following clean-up by extraction with ethylacetate and column chromatography. 3-Hydroxypropylmercapturic acid (HPMA) was quantified with an S-specific detector after a similar clean-up procedure. Hydroxyphenanthrenes and hydroxypyrenes in urine were analysed by enzyme hydrolysis, extraction into *n*-hexane and clean-up by HPLC fractionation prior to detection as silylated derivatives using GC/MS. Urinary mutagenicity was determined as previously described using the *Salmonella typhimurium* (TA 98)/microsome assay [62]. DNA adducts in lymphocytes were determined by the ^{32}P-postlabelling assay as previously described [24]. Lymphocytes were isolated from the supernatant of white blood cell cultures [24]. DNA binding substances (DABS) in the particle fractions of MS and ETS as well as in urine extracts were detected by ^{32}P-postlabelling of digested DNA isolated from in vitro incubations (37° C, 1 h) of calf thymus DNA with hepatic S9-mix of aroclor-induced rats and cofactors [5, 64].

Statistical analysis

Paired samples (exposed vs non-exposed) were analysed using the Student's *t*-test. For blood and plasma parameters, the level in the respective morning sample before the start of exposure was used as the non-exposed reference value. Similarly, 24-h urine of the previous control day was used as the non-exposed reference value.

Results and discussion

Exposure levels

The results for air monitoring in the experimental room in study 2 are shown in Table 3. ETS components are divided into particle-bound and gas-phase constituents. Except for gas-phase phenanthrene and pyrene, significant increases were measured on the days with experimental ETS exposure (day 3 and 5) as compared with the sham-exposure days (days 2 and 4). The concentrations for the most frequently used ETS markers such as RSP (3–4 mg/m^3), CO (24 ppm) and nicotine (71 μg/m^3) were 10 times higher than those found in everyday environments where real-life exposure to ETS may occur. For example, typical RSP concentrations in real-life rooms where smoking takes place range from 0.05 to 0.35 mg/m^3 [44, 67].

Table 3. Air monitoring in the experimental room during study 2. Data are time-weighted averages for the 8-h exposure sessions. Days 2 and 4 were sham-exposure sessions, and during day 3 and day 5 sessions a total of 120 cigarettes were smoked

	Day			
	2	3	4	5
Particulate matter				
RSP (μg/m^3)	77	3181	78	4091
BaP (ng/m^3)	0.2	21.5	0.3	26.7
BeP (ng/m^3)	0.8	21.5	0.8	24.9
Coronene (ng/m^3)	0.1	2.8	0.6	2.2
Anthanthrene (ng/m^3)	0.06	3.9	0.07	3.1
Benzfluoranthenes (b+j+k) (ng/m^3)	2.1	52.3	1.7	55.3
Chrysene (ng/m^3)	1.8	54.2	1.5	70.5
BaA (ng/m^3)	2.1	18.9	1.1	26.2
Phenanthrene (ng/m^3)	3.7	6.8	1.8	7.4
Pyrene (ng/m^3)	0.6	17.6	0.7	20.5
NNN (ng/m^3)	1	4	1	5
NNK (ng/m^3)	1	9	1	6
Gaseous phase				
CO (ppm)	1.4	24	2.0	24
NO_x (ppb)	38	422	56	449
Formaldehyde (μg/m^3)	3	48	3	49
Acetaldehyde (μg/m^3)	290	1450	85	1390
Propionaldehyde (μg/m^3)	15	120	14	120
Nicotine (μg/m^3)	4	71	6	71
Benzene (μg/m^3)	8	190	12	206
Phenanthrene (ng/m^3)	138	154	nd	258
Pyrene (ng/m^3)	29	24	nd	25

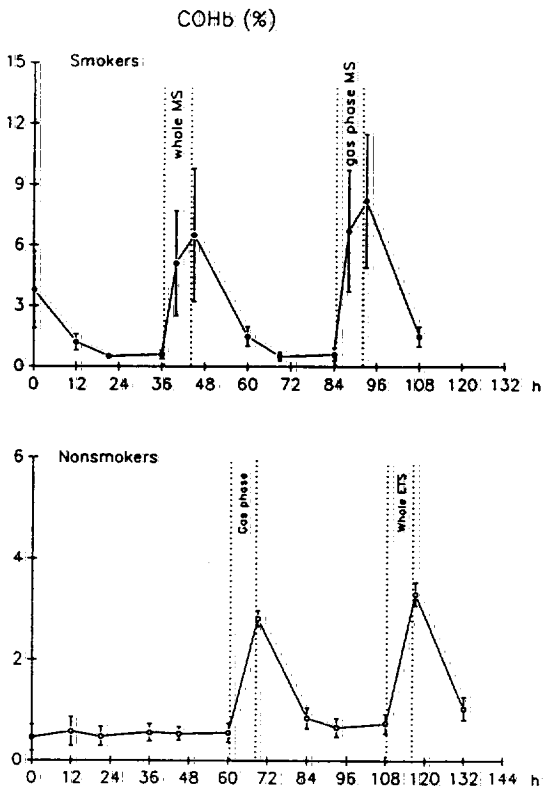

Fig. 1. Time-courses of carboxyhaemoglobin (COHb) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

Therefore, ETS exposure levels in our studies have to be regarded as purely experimental.

More recently, 3-vinylpyridine has been proposed as a gas-phase marker for ETS [11] and solanesol as a particle-phase marker for ETS [44]. In study 5 we measured both ETS components. 3-Vinylpyridine concentrations were 0 and 15 $\mu g/m^3$ on the control and exposure days, respectively. For solanesol the respective levels were 0 and 110 $\mu g/m^3$. Solanesol concentrations under real-life conditions range form 6.4 to 12.8 $\mu g/m^3$ [44].

Biomonitoring

In the following sections the uptake of different tobacco smoke constituents after experimental ETS exposure are compared with those after active smoking. For some substances, the experimental design allows differentiation between uptake as gas-phase or as particle-bound substances [62].

Carboxyhaemoglobin (COHb). The time-courses of COHb for smokers and ETS-exposed non-smokers are shown in Fig. 1. As expected, increases after gas-phase and whole MS or ETS exposure are similar. The reasons for the somewhat higher COHb levels after gas-phase MS smoking as compared with whole MS smoking and whole ETS exposure as compared with gas-phase ETS exposure have been discussed previously [62]. Under the experimental conditions applied, the mean increase in COHb after smoking (6.5–8.2%) is three times larger than that after ETS exposure (2.5%), which is in agreement with the estimated uptake ratio (Table 2) considering that the experimental conditions involved extremely high exposure to ETS containing 24 ppm CO for 8 h. Under real-life ETS exposure, no increase, or only a marginal increase, in COHb concentration is observed [29, 68], which again demonstrates that extremely high ETS doses were applied. Due to its low sensitivity and lack of specificity, COHb is not a suitable biomarker for everyday ETS exposure.

Nicotine and cotinine. The uptake of nicotine was monitored by determining nicotine (Fig. 2) and cotinine (Fig. 3) in both plasma and urine. The results clearly show that smokers take up nicotine in particle-bound form. In contrast to this, nicotine is taken up as a gas-phase constituent from ETS (Fig. 2). Due to the reduced capacity of non-smokers to metabolize nicotine to cotinine and the long biological half-lives of both these compounds, higher amounts of cotinine are found in plasma and urine on the days after exposure of non-smokers to gas phase and whole ETS than on the actual exposure days (Fig. 3). These findings are in agreement with analytical data showing the nicotine in MS is particle-bound, whereas it is nearly exclusively found in the gas phase of ETS [14]. Therefore, nicotine and cotinine in body fluids are biomarkers for the uptake of smoke particles in active smokers but indicate ETS gas phase exposure in passive smokers. Comparison of cotinine levels in body fluids of active and passive smokers for the purpose of risk estimation, as performed by Russell [56], is therefore misleading. On the other hand, cotinine in plasma, urine and saliva is presently the most reliable biomarker for ETS exposure [29], as long as comparisons are limited to subjects with different degrees of ETS exposure. Extrapolation from the uptake of nicotine to that of other tobacco smoke constituents is of doubtfull validity due to a more rapid removal of nicotine than other components from ETS in indoor environments [11].

Thioethers (mercapturic acids). The urinary excretion of thioethers is regarded to be an indicator

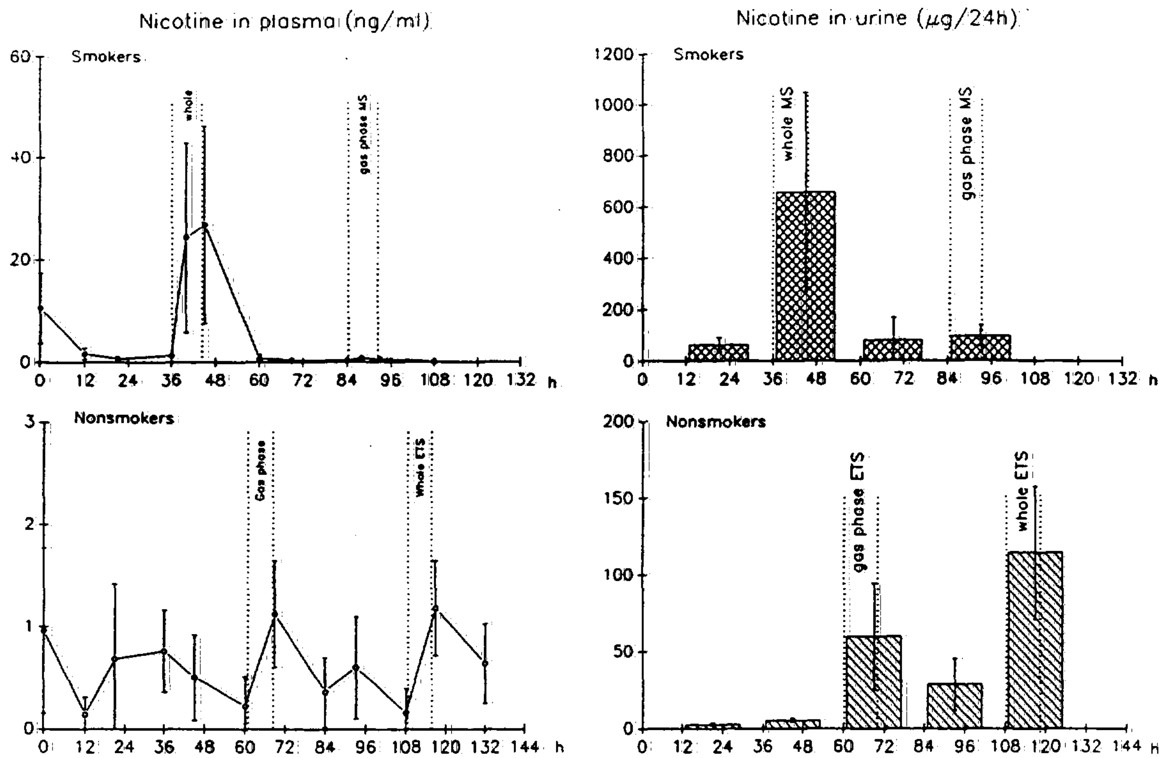

Fig. 2. Time-courses of plasma nicotine (*left*) and urinary excretion of nicotine (*right*) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

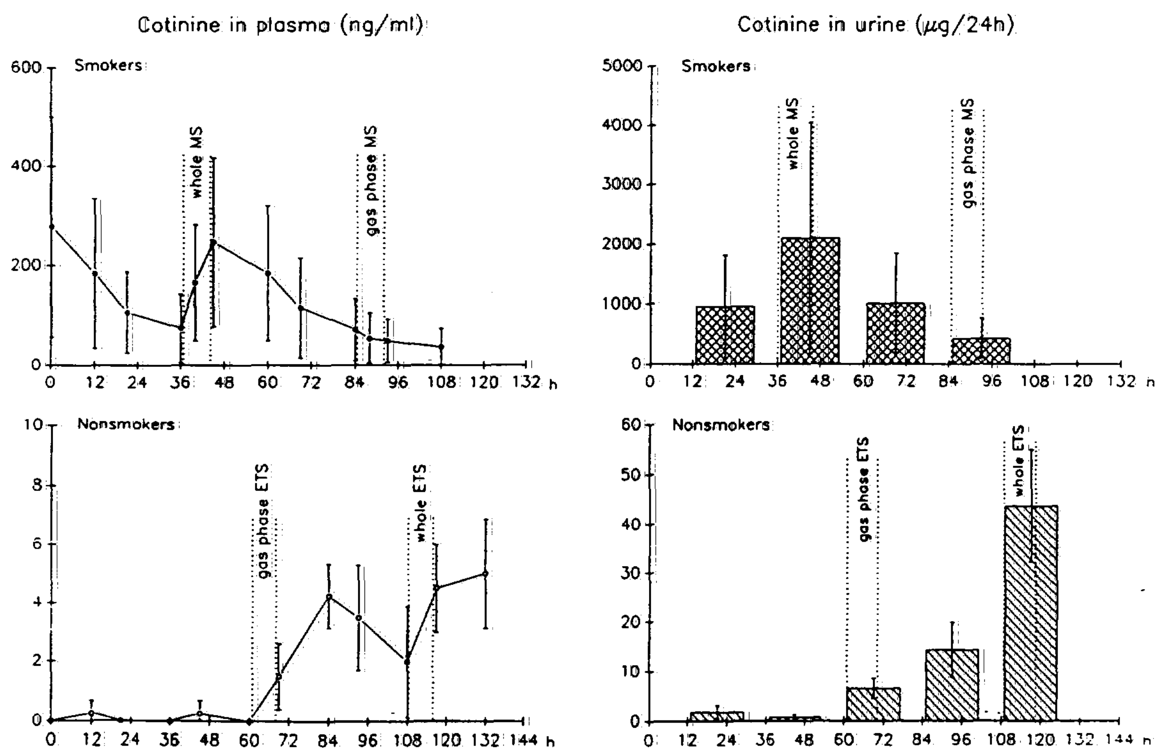

Fig. 3. Time-courses of plasma cotinine (*left*) and urinary excretion of cotinine (*right*) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

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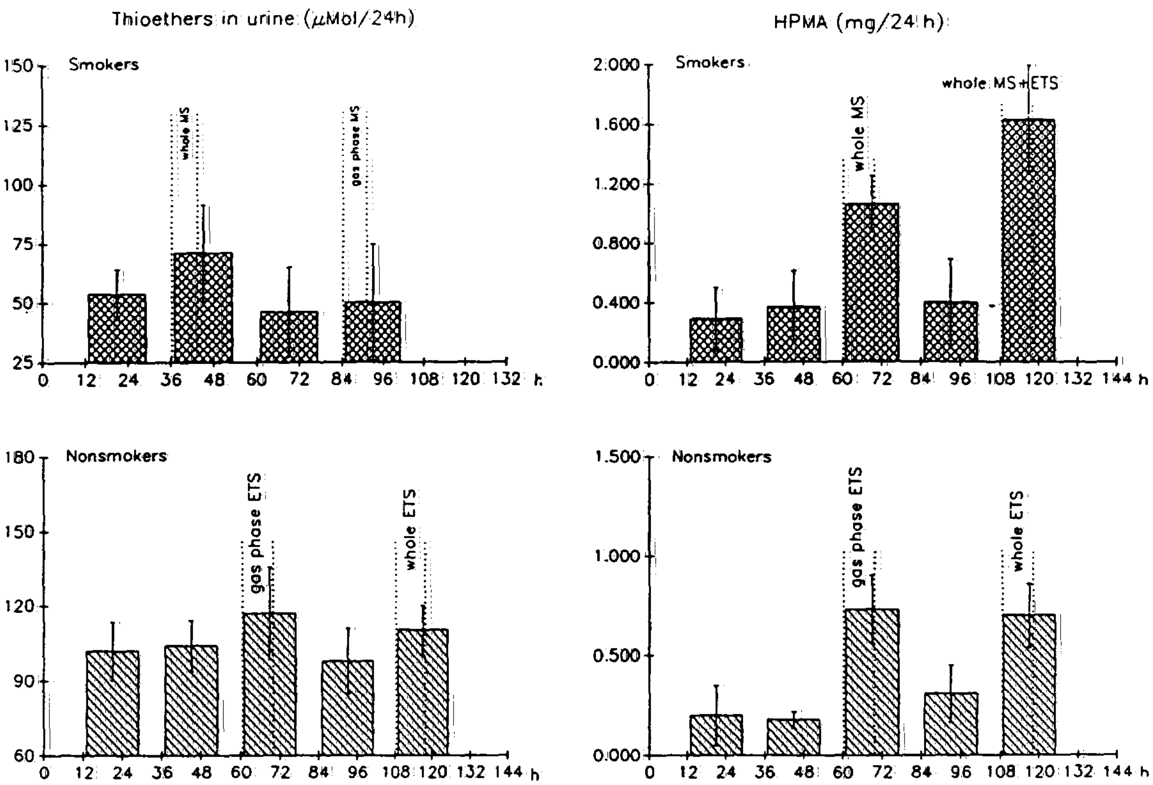

Fig. 4. *Left.* Urinary excretion of thioethers by smokers (study 1) and non-smokers (study 2). *Right.* Urinary excretion of 3-hydroxypropylmercapturic acid (HPMA) by smokers (study 2) and non-smokers (study 2). Data are means with standard deviation bars

of the exposure to electrophilic substances [6]. The intracellular glutathione-S-alkyl-transferase/-glutathione system protects the organism from the destructive effects of electrophiles by binding them to the free SH-group of glutathione. Smoking has been shown to increase thioether excretion [71]. We have confirmed this in a previous study [61]. In addition, we also found a significant increase in thioether excretion by non-smokers after high ETS exposure. In more recent studies [62], we showed that the elevated thioether excretion by smokers is primarily caused by uptake of MS particles, whereas in ETS-exposed non-smokers the increase in thioether excretion is due to only the gas phase of ETS (Fig. 4).

The tobacco smoke constituents responsible for thioether formation are unknown. In a first attempt to characterize and identify tobacco smoke-related thioethers, we profiled the sulphur-containing acids (assumed to contain the majority of mercapturic acids) in the urine of smokers and non-smokers with and without smoking or ETS exposure, respectively. In most of the subjects, the number and intensity of the S-containing peaks in the gas chromatograms increased after tobacco smoke exposure. However, identification of specific mercapturic acids turned out to be very difficult in these complex mixtures. We were able to identify 3-hydroxypropylmercapturic acid (HPMA) in urine of smokers and ETS-exposed non-smokers. The elevation of HPMA in urine of passive smokers is exclusively caused by the gas phase of ETS (Fig. 4). No assertion for smoking with respect to HPMA being formed by gas- or particle-phase constituents of MS can be made, since HPMA was not determined in study 1. It is not clear which tobacco smoke constituents are responsible for the in vivo formation of HPMA. After application of acrolein to rats, HPMA has been reported to be a major metabolite in urine [32]. However, this was not confirmed in a more recent investigation [10].

The elevation of non-specific urinary thioethers following exposure of active smokers to particulate matter and passive smokers to gas-phase ETS again shows that differences exist between active

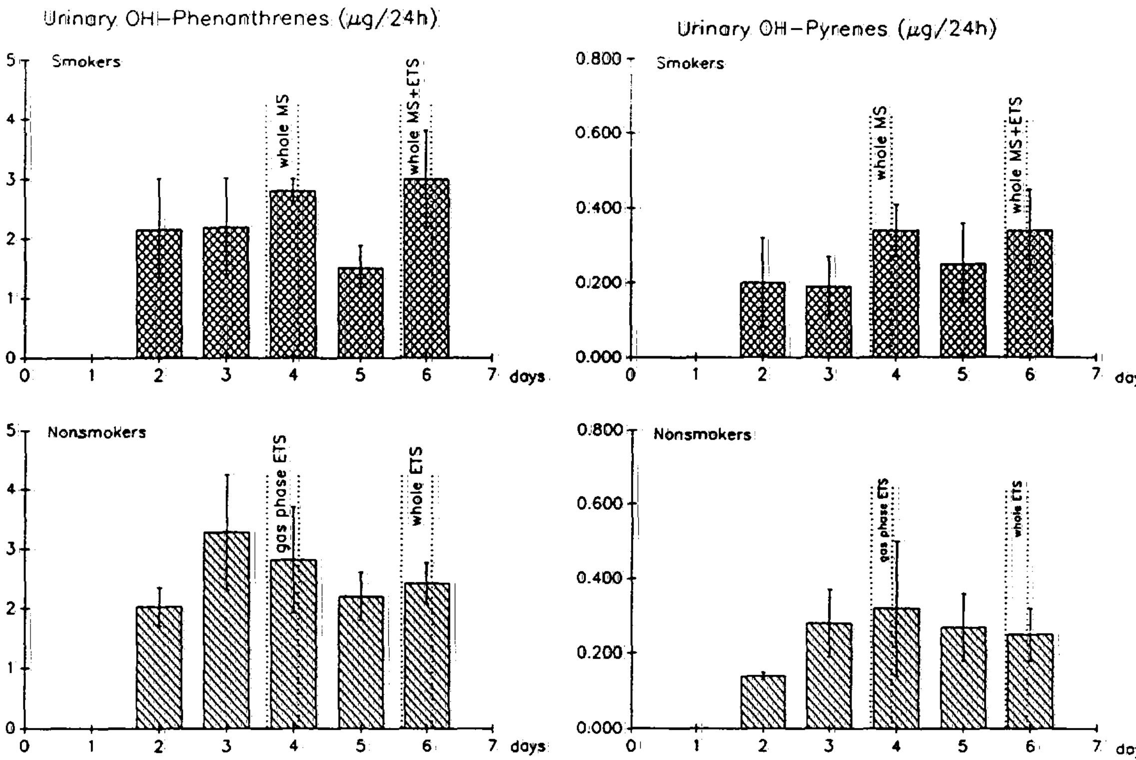

Fig. 5. *Left.* Urinary excretion of hydroxyphenanthrenes by smokers (study 2) and non-smokers (study 2). Hydroxyphenanthrenes are the sum of 1-, 2-, 3-, 4- and 9-OH-phenanthrene. *Right.* Urinary excretion of hydroxypyrenes by smokers (study 2) and non-smokers (study 2). Hydroxypyrenes are the sum of 1- and 2-OH-pyrene. Data are means with standard deviation bars

smoking and exposure to ETS, and an extrapolation from active to passive smoking cannot be made.

In general, the determination of urinary thioethers is not a suitable method for biological monitoring of tobacco smoke exposure. Apart from the low sensitivity and the lack of specificity, the main reason is that changes in thioether excretion can only be detected when the diet is carefully controlled as performed in our studies [2, 61, 62].

Polycyclic aromatic hydrocarbons (PAH). PAH represent a class of carcinogens in tobacco smoke which has been intensively investigated. Their importance in tobacco smoke carcinogenesis has previously been rated high [18]. More recently, other carcinogens, such as nitrosamines and free radicals, as well as cocarcinogens and promoters, are assumed to be of similar, or even greater, importance in tobacco smoke carcinogenesis [26]. Hydroxyphenanthrenes [23] and hydroxypyrenes [30] in urine have been proposed as biomarkers for the uptake of PAH. Phenanthrene is primarily a gas-phase constituent of ETS, the background concentration is relatively high as can be seen from the level of 138 ng/m^3 observed on day 2 of study 2, when no smoking took place in the experimental room (Table 3). ETS exposure, either with gas-phase or whole ETS, does not lead to a measurable increase in urinary hydroxyphenanthrene excretion (Fig. 5). After smoking, a small but significant increase was found (Fig. 5). In everyday passive smoking, ETS-related uptake of phenanthrene (and PAH in genral) can be neglected, particularly when the diet as an important source for PAH uptake is considered [39].

The same conclusion can also be drawn from the findings with urinary hydroxypyrene excretion after smoking and ETS exposure (Fig. 5). A small but significant increase is observed after smoking. The room air measurements show pyrene concentrations in the gas phase of about 25–30 ng/m^3, which are unrelated to the actual smoking taking place in the room (Table 3). The particle-bound pyrene levels increased from <1 ng/m^3 without smoking to 18–20 ng/m^3 with smoking (Table 3). However, this increase in particle-bound pyrene did not lead to a measurable elevation in urinary

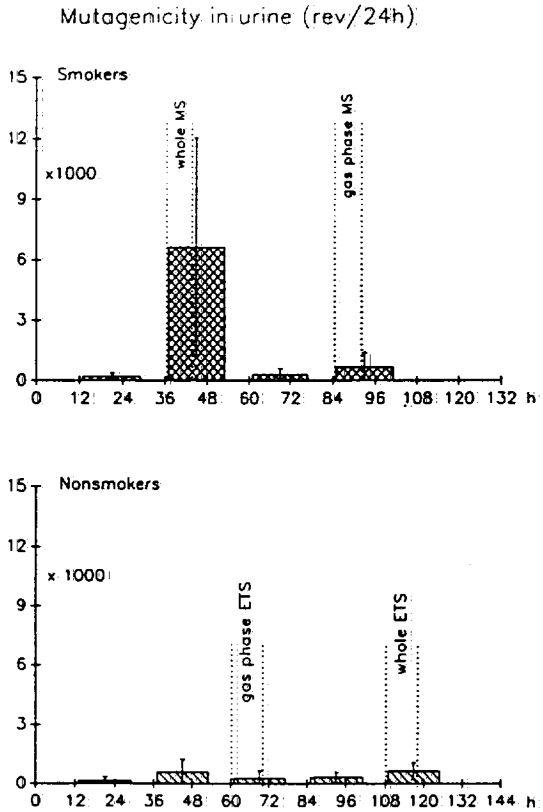

Fig. 6. Urinary mutagenicity in smokers (study 1) and nonsmokers (study 2). Data are means with standard deviation bars

hydroxypyrene excretion after whole ETS exposure, indicating that either the background body burden of pyrene was too high and/or uptake of ETS particle phase was too low. The latter explanation is in accordance with the low deposition rate of ETS particles in the respiratory tract during passive smoking [21].

Determination of PAH metabolites in urine is not sensitive enough for biomonitoring of ETS exposure under real-life conditions. The data shown here and those from our earlier studies [1, 23, 29] suggest that passive smoking only marginally contributes to the everyday PAH exposure. General air pollution and diet are the major exposure sources to PAH.

Mutagenicity. A more general approach for determining the uptake of genotoxic substances is the measurement of urinary mutagenicity in exposed subjects. Increased mutagenicity in smokers' urine has been found in many studies [26]. Our results with active smokers clearly show that the urinary mutagenicity is caused nearly exclusively by the uptake of MS particulate matter (Fig. 6). Whether ETS exposure can lead to an elevated urinary mutagenicity is a matter of controversy. In most investigations no significant increase has been observed [25, 31, 60, 66]. In two studies [4, 40], with experimental ETS exposures comparable to those in our investigations [60, 62], a significant elevation in urinary mutagenicity was found. In study 2, urinary mutagenic activity after gas-phase and whole ETS exposure were within the normal fluctuations of the method (Fig. 8) [62]. There is sufficient evidence that ETS particles are mutagenic [38]. In ETS about 10% [57], and in sidestream smoke (SS) about 20% [7], of the mutagenic activity has been found in the vapour phase. Therefore, it can be assumed that the mutagenic activity of ETS is primarily particle bound.

Based on the particle concentration occurring during experimental ETS exposure (Table 3), a breathing rate of 0.5 m^3/h and a deposition rate of 11% [21], it can be estimated that the nonsmokers in study 2 could have taken up about 1.8 mg of ETS particles during exposure on day 5 compared with about 300 mg of MS particles taken up the smokers smoking 24 cigarettes. Assuming similar specific mutagenic activities for ETS and MS particles (MS particles have been reported to have a specific activity 2–3 times higher than SS particles) [7], ETS exposure in terms of particle exposure would be equivalent to smoking 0.14 cigarettes. In our experience, the Ames test for detection of urinary mutagenicits is far too insensitive to assess such a low exposure. The results of our investigations [60, 62], as well as those of other authors [25, 31, 66], suggest that urinary mutagenicity, which would be a potential marker for ETS particle exposure, remains unchanged after ETS exposure. Taking into consideration dietary factors that may elevate or reduce the mutagenic activity of urine [1, 23, 39, 58], we conclude that this is not a useful marker for the uptake of genotoxic substances in real-life passive smoking.

DNA adducts. DNA adducts are useful biomarkers for the detection of exposure to genotoxic substances at the relevant target, namely the DNA in the cell nucleus. DNA adduct levels are modulated by several factors including exposure dose, metabolic activation and detoxification, survival time of the cells under investigation, as well as stability and repair of the formed DNA adducts. Determination of DNA adducts by the ^{32}P-postlabelling assay is a sensitive method with a detection limit of 1 adduct/10^{10} normal nucleotides [52].

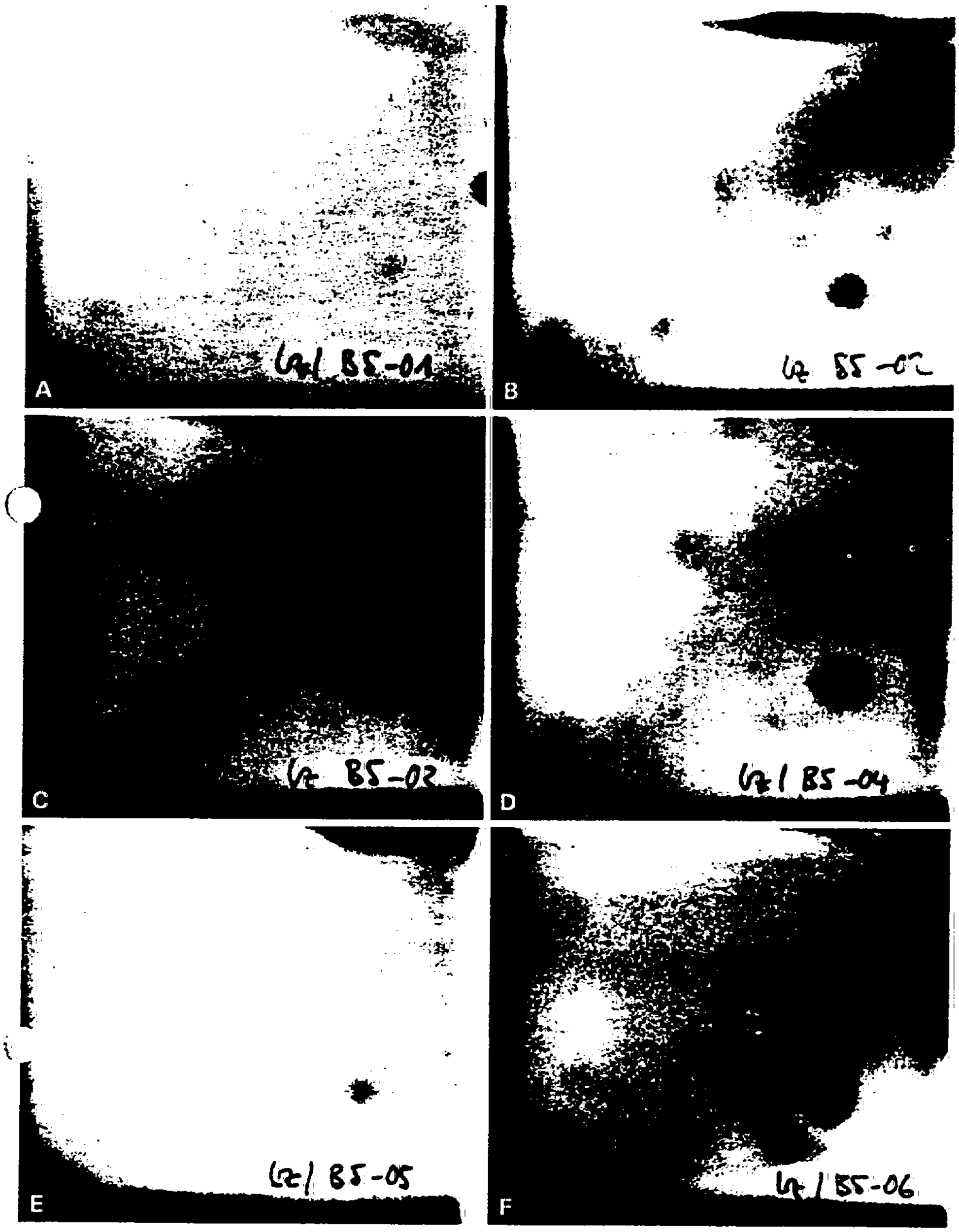

Fig. 7. Autoradiograms of ^{32}P-postlabelled DNA adducts from lymphocytes of five smokers (A–E) and five non-smokers (F–J). Blood samples for lymphocyte isolation were drawn directly after the exposure session on day 2 of study 2. Autoradiography was carried out at −84° C for 100 h

Since the chemical structure of the formed adducts does not need to be known, this method is particularly useful for the detection of DNA adducts after exposure to complex mixtures such as tobacco smoke. The ^{32}P-postlabelling method was applied to DNA of blood monocytes of the subjects in Study 2 [24]. Additional smoking-related DNA adducts were found after smoking 24 cigarettes in 8 h. These adducts disappeared within 36 h after smoking cessation, which is primarily due to the limited life-span of monocytes (about 8 h). No additional adduct spots could be detected after ETS exposure [24]. In a recent study (study 3) with similar tobacco smoke exposures, exposure-related ad-

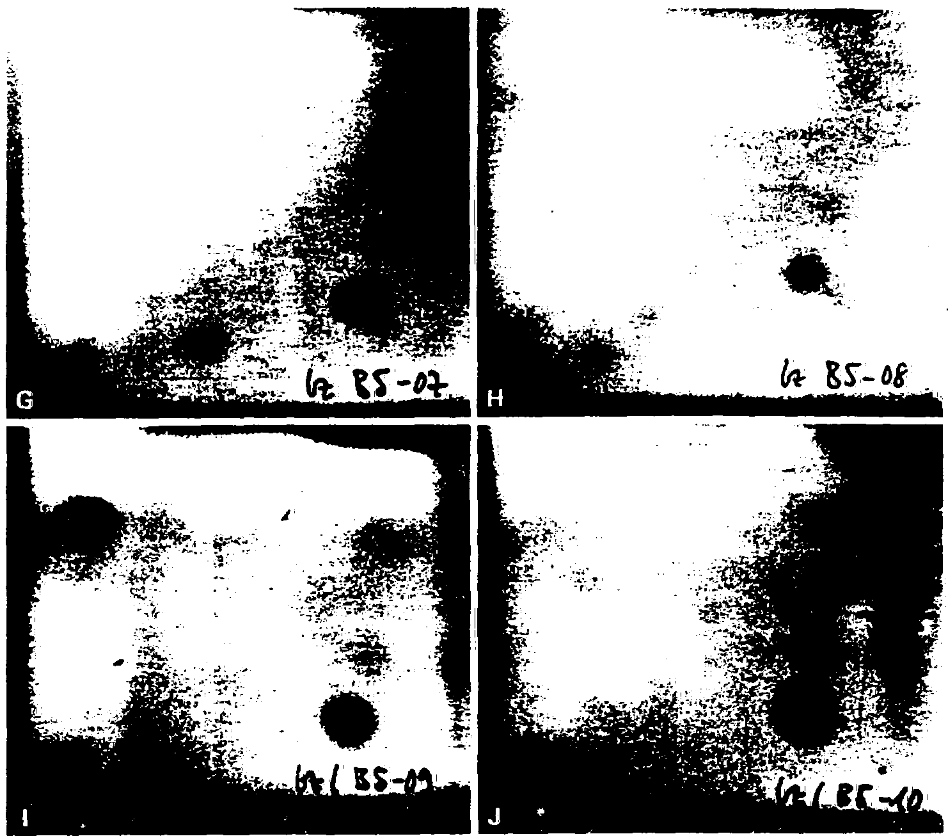

Fig. 7G–J

ducts could not be identified in monocytes from smokers and non-smokers [63]. However a slightly greater number of adducts were found in monocytes of smokers when smoking the complete MS as compared with smoking the gas phase of MS or being abstinent from smoking. Although these results need further confimation, they are in agreement with the idea that the ^{32}P-postlabelling assay with nuclease P1 enrichment detects bulky aromatic adducts, most probably originating from particle-phase constituents of tobacco smoke. The lack of additional DNA adducts in ETS-exposed non-smokers suggests that the uptake of ETS particles is below the detection limit of this method.

In contrast to DNA adducts in monocytes, DNA adducts in T lymphocytes ($\sim$80% of total lymphocytes), which have a life-span of several years, should indicate chronic exposure to DNA adduct-forming compounds. The autoradiograms of five smokers and five non-smokers are shown in Fig. 7. Visual inspection of the spot patterns reveals no characteristic difference between smokers and non-smokers. This qualitative result is in agreement with other reports from the literature [27, 49, 50, 53, 59]. In one study [59], the mean adduct levels in smokers were found to be significantly higher than those in non-smokers. In the same study, a significant intra-individual variation (two blood samples from three smokers were drawn with a 3-week interval) was observed. We made the same observation when analysing four blood samples drawn within 4 days from each subject in study 3.

In our view, the detection of tobacco smoke-related DNA adducts in white blood cells by the ^{32}P-postlabelling method has not been able to reveal conclusive results. We agree with Foiles et al. [16], who regard the lack of information on the structure of the majority of adducts observed by this method in different human tissues as a serious limitation.

In another approach, we tried to detect 'DNA binding substances' (DABS) in complex mixtures such as MS particulate matter, ETS particles and urine extracts of smokers and non-smokers with and without tobacco smoke exposure [64]. These mixtures were incubated with a target DNA and a metabolizing system so that the direct carcinogens and the enzymatically formed ultimate carcinogens could bind to target DNA. It has been sug-

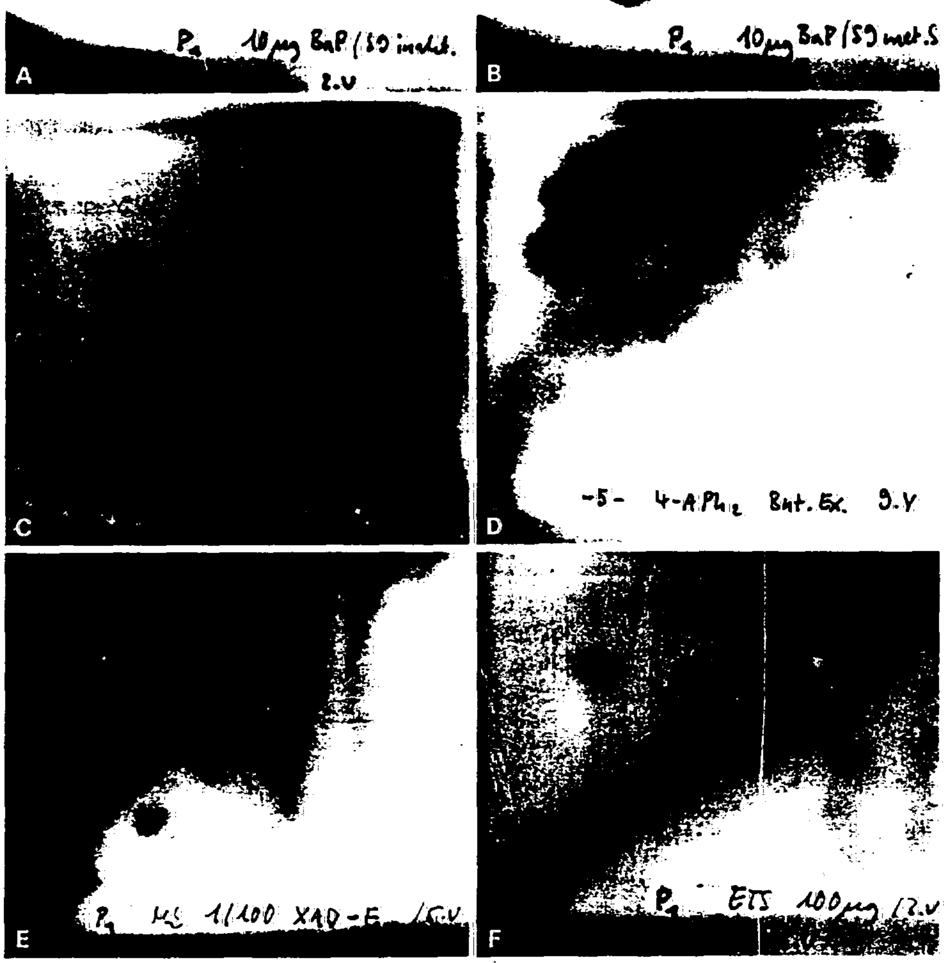

Fig. 8. Autoradiograms of ^{32}P-postlabelled DNA adducts formed after in vitro incubation of benzo(a)pyrene (BaP) (A, B), 4-aminobiphenyl (4-ABP) (C, D), particle phases of mainstream smoke (*MS*) (E) and environmental tobacco smoke (*ETS*) (F) as well as extracts of 100 ml urine from smokers (G, H) and non-smokers (I–L) collected in study 4. All samples except for sample A were incubated with active S9-mix as described in the text. Adduct enrichment was performed by either nuclease P1 treatment (P1) or butanol extraction (BE) as indicated. Autoradiography was carried out at −84° C for 40 h. A BaP (10 µg)+ inactivated S9-mix; B BaP (10 µg)/P1; C 4-ABP (50 µg)/P1; D 4-ABP (50 µg)/BE; E MS (1/100 cigarette) P1; F ETS (100 µg)/P1; G urine extract of a smoker/P1; H same as G/BE; I urine extract of a non-smoker without ETS exposure/P1; J same as I/BE; K urine extract of non-smoker I after ETS exposure/P1; L same as K/BE

gested that the mutagenic compounds in smokers' urine are heterocyclic aromatic amines [13, 47] of which 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) has been implicated as a major cause of DNA damage [48]. A metabolizing system able to activate these compounds was used [5]. Results obtained with this in vitro assay are shown in Fig. 8. The particulate phases from MS and ETS

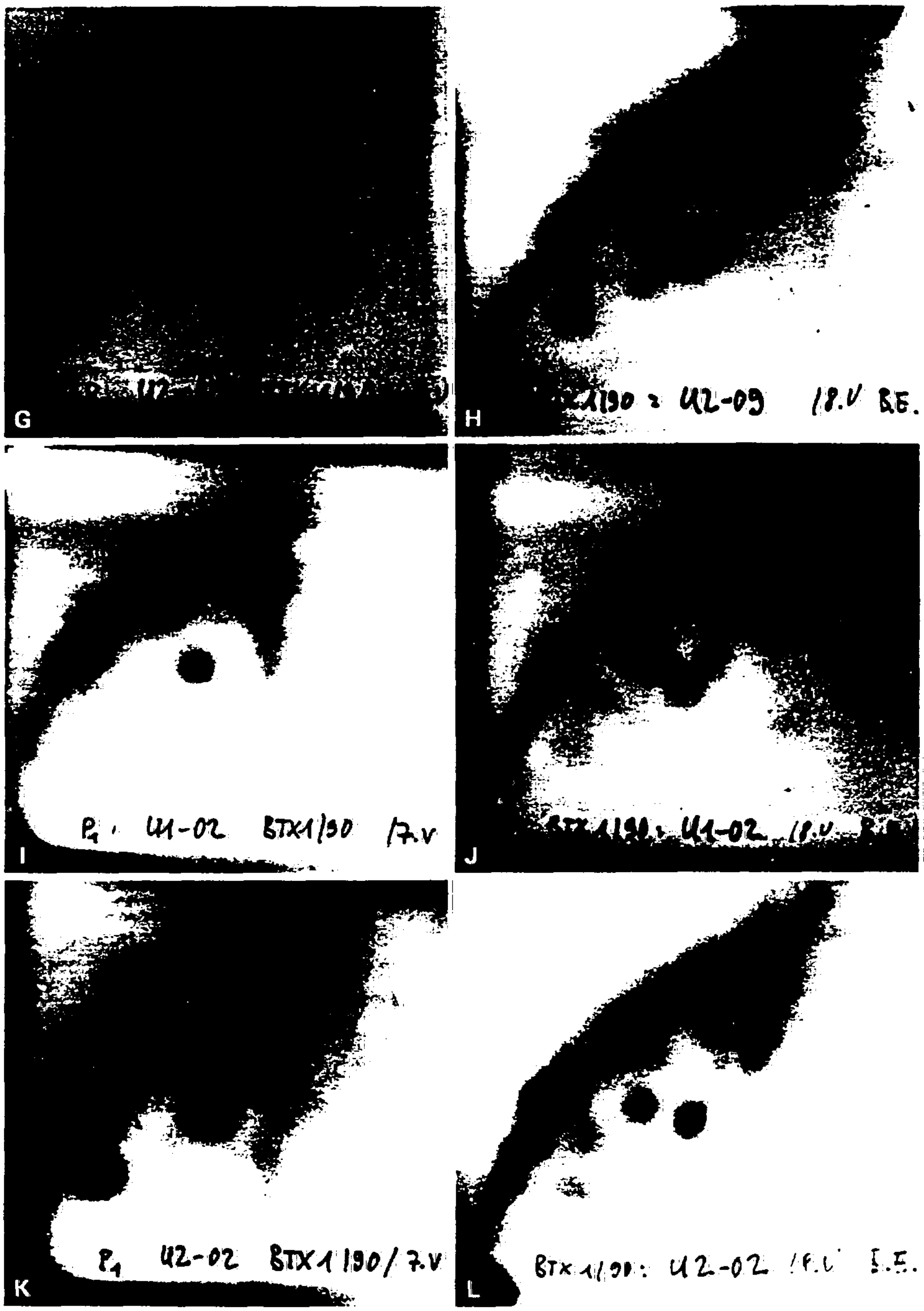

Fig. 8G–L

form diagonal radioactive zones (DRZ) comparable to those observed with DNA from lung, heart, kidney, bladder and oesophagus from smokes [51].

Urine extracts from smokers and non-smokers (either with or without tobacco smoke exposure) showed a couple of DNA adduct spots. The spot pattern varied with the adduct enrichment method (nuclease P1 treatment or butanol extraction). The detected spots were not related to one of the heterocyclic aromatic amines tested in this assay. Fur-

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thermore, no DNA adducts could be identified which were exclusively related to smoking or ETS exposure. Since the diet was carefully controlled in these studies, it is unlikely that the observed inter-individual differences in urinary DABS were caused by dietary factors. These results do not agree with those reported by Peluso et al. [47, 48], who found no, or only faint, DNA adduct spots in urine extracts of non-smokers, and adduct spots in urine extracts from smokers of black tobacco suggested to have been caused by PhIP. Investigations are in progress in our laboratory in order to clarify the origin of the DABS in both smokers' and non-smokers' urine extracts.

Conclusions

he small increase in lung cancer risk in non-smokers exposed to other people's tobacco smoke, as suggested by meta-analysis of more than 20 epidemiological studies, is at variance with dosimetric findings observed in exposure studies with smokers and non-smokers. These studies have shown that the uptake of particle-bound substances is predominant in active smoking, whereas uptake of gas-phase substances is predominant in passive smoking. According to present knowledge, the genotoxic potential is primarily located in the particulate matter of both MS and ETS. Currently available biomonitoring methods sensitive enough for detecting ETS exposure only indicate the uptake of gas-phase constituents of ETS. There is no biomarker available which indicates the uptake of ETS particles. Therefore, although low amounts of genotoxic substances can be inhaled by passive smoking, a risk evaluation on the basis of dosimetic data for these compounds is not presently possible.

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Environmental tobacco smoke (ETS) retention in humans from measurements of exhaled smoke composition.

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ABSTRACT

Twelve male volunteers were exposed for 60 minute periods to two concentrations of aged and diluted sidestream tobacco smoke (150 and 920 $\mu g.m^{-3}$ particulate), generated from a cigarette type, representative of middle-tar brands available in the UK, and at concentrations (for the lower level), similar to those reported from environmental surveys. Twelve female volunteers were exposed at the high level only. Measurements were carried out using an inhaled - exhaled technique.

For the twelve male volunteers, mean retention and standard deviation of particulate material as measured by UVPM (UV absorbance at 325 nm) the high and low exposure levels respectively were 41 $\pm$ 14% and 36 $\pm$ 20% (95% CI (higher level) : 30 - 50%), consistent with data from radiotracer studies (43 $\pm$ 17%) and from computer models of lung deposition for measured breathing patterns (34 $\pm$ 8%). Mean solanesol retention at the higher level exposure was 40 $\pm$ 20% (95% CI : 30 - 50%). Mean nicotine retention at the high and low exposure levels was 77 $\pm$ 17 % and 71 $\pm$ 12 % (95% CI : 62 - 88%), significantly greater than particulate retention.

For women, mean nicotine retention was 81 $\pm$ 16 % (95% CI : 70 - 91%), consistent with male data, but ETS particulate retention was significantly lower when measured by UVPM (17 $\pm$ 10% : 95% CI : 10 - 23%). Particulate retention as determined by solanesol measurements (27 $\pm$ 14% : 95% CI : 14 - 40%) was not significantly different from male values. The female particulate retention data were in agreement with model predictions of retention for measured breathing patterns (26 $\pm$ 5%).

High inter-subject variation was observed for both men and women for all three markers. In addition, the ratio of airborne particles to nicotine in the exposure chamber was variable

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with concentration, which is consistent with data from environmental surveys. It is concluded that these variations preclude calculations of particulate retention when only environmental nicotine concentrations are known, or when nicotine exposure has been extrapolated from biomarker data for nicotine or cotinine (a major nicotine metabolite).

INTRODUCTION

Human exposure to environmental tobacco smoke (ETS) cannot be defined adequately in terms of exposure to airborne concentrations of ETS components alone as this is only one of many factors which affect the retention, and hence dose of these smoke components (McAughey *et al*, 1990). One of the most important of these additional factors is the degree of deposition of ETS particulate in the lung relative to more volatile ETS components. Using available data, particle deposition in the tracheo-bronchial region of the lung in passive smokers has been estimated as 0.02% that of mainstream smokers (McAughey *et al*, 1989), although this is dependent on the assumptions used and does not account for differences in chemical composition of the two smokes. One of the most important variables in this calculation is the fractional and regional deposition of particulate in the lung. The commonly used value of 10-11% total respiratory tract deposition is based on a single series of experiments by Hiller (1984). However, the measurement technique employed required that high concentrations of sidestream smoke be maintained, which was not representative of typical environmental exposures to ETS, where ageing and dilution effects are important in achieving an equilibrium ETS aerosol.

Nicotine and one of its principal metabolites, cotinine, have frequently been used as biological markers of ETS exposure. However, the physical and chemical behaviour of nicotine in ETS is not representative of other ETS components, and therefore, nicotine is likely to be a poor general marker (Eatough *et al*, 1989). This is confirmed by a recent publication which shows that the ratio of nicotine to both vapour- and particulate-phase components is highly variable (Nelson *et al*, 1992). A study by Iwase *et al* (1991) has measured retention of nicotine from ETS and shown retention fractions of 60 - 80 % (mean $\pm$ SD : 71.3 $\pm$ 10.2%) in a group of 17 non-smoking women inhaling sidestream

smoke via an 'inhaled' minus 'exhaled' technique. However, particulate retention was not measured.

This study has enlarged the limited database of the proportion of the inhaled particulate phase of ETS which is retained by non-smokers, by measuring retention of specific components from aged and diluted sidestream smoke in male and female volunteers. The technique involves subtraction of exhaled quantities from quantities of the respective components inhaled. The measured components of the smoke aerosol were nicotine, UVPM (UV absorbance of a methanolic extract at 325 nm - a marker of ETS particulate (Conner *et al*, 1990)), and solanesol (a primary trisesquiterpenoid alcohol specific to the lipid fraction of tobacco leaves), which remains associated with ETS particulate as sidestream smoke ages and is diluted (Ogden and Maiolo, 1988). Exposures of all male subjects were carried out at two concentrations of ETS, the lower similar to values reported from environmental surveys, and a higher for improved confidence of analytical results above detection limits. Data from female subjects are available from exposures at the higher level only, as exhalate samples for UVPM and solanesol from the lower exposure concentration were below analytical detection limits, due to a combination of low ventilation rates in the female subjects.

MATERIALS AND METHODS

Environmental Chamber

Atmospheres containing a surrogate ETS (aged and diluted sidestream smoke) were generated in a 14 m^3 chamber, in which temperature and humidity were controlled, and in which smoke concentrations were maintained at steady-state for 3 hours.

Smoke Generation

A detailed description of the smoke generation facilities may be found in Black *et al*, (1987). In brief, smoke was generated in a 0.4 m^3 glass-fibre glove box using a Mason-BAT smoking machine (Bevan, 1976) capable of smoking 1 to 12 cigarettes on a continuous cycle. The cigarette type used was typical of UK middle-tar brands. Sidestream smoke generated from the cigarettes was swept continually into the chamber by air diverted from the main inlet to the room, and then recombined with the main flow (Figure 1). Air from the glove box can also be fed directly into the laboratory extract, via a filter, to allow continuous control of the resulting smoke concentration. By varying the number of cigarettes, dilution of the smoke, and the air flow through the room, a wide range of concentrations can be achieved which represent a mix of fresh and aged smoke. Once equilibrium of carbon monoxide and particulate levels has been attained, volunteers can be introduced into the chamber via an airlock, thereby permitting exposures under constant conditions. Previous work has shown spatial mixing within the room to be good with a coefficient of variation for CO and particulate concentration at twelve sites within the room of 6.5% (Pritchard *et al*, 1988a).

Exhaled Breath Capture System (Valving)

The system for collecting exhaled breath was based upon that developed for the exposure of volunteers to radioactive aerosols (Walsh *et al*, 1978 - Figure 2), although smaller exhalate filters were used in this study (90 mm reduced to 47 mm). The valving system comprised a breath (pressure) actuated Teflon™ shuttle valve, which moves between an inhale and exhale position. Breathing frequency, flow and volume were monitored on the inlet line via a pneumotachograph, with data handling and storage on a PC microcomputer. The exhalate line was attached, via an exhalate filter pack, to an external suction pump across a diaphragm valve, actuated by a pressure feedback loop, designed such that the volunteer felt no resistance to exhalation. The valve assembly was suspended from a counter-balanced pulley within the chamber, to permit the volunteers some freedom of movement. The mouthpiece of the valving assembly was modified to take an oro-nasal mask, part of which was occluded in order to reduce the dead-space in the breathing volume. Dead-space in the oro-nasal mask and valving assembly introduces potential errors into the measurement of retention, because the last part of an inhalation draws material into the valve assembly which never enters the respiratory tract. As soon as exhalation commences, this material is blown onto the filter. Similarly, at the start of inhalation, the initial material inhaled is the last part of the exhalate, which could be at a substantially lower particle and nicotine concentration than the chamber air. Thus, if the dead-space is large compared to the tidal volume, significant errors in the estimated retention occur. However, the effect of dead-space, and also any deposition in the valve assembly was accounted for mathematically. It has also been noted that during the finite time the valving system switches from inhale to exhale, some material may be re-exhaled through the inlet tube rather than to the exhale filter. This was measured and logged and was considered in the deadspace calculations for each subject.

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Mask fitting was checked during trial exposures, with the mask used having an air-filled sealing region to help fit by moulding to the face and the subjects taught how to check the seal. However, leakage remains a possibility during these long exposures. This was minimised by adjusting system flows across the exhalate diaphragm valve so that no resistance to exhalation was offered, and by being able to adjust suction during the exposure if the subject was aware of resistance changes on exhalation.

Exhale Filter Pack

The filter pack for the collection of exhalate comprised a 47mm diameter Teflon™ filter (Millipore, UK) to trap particulate material for subsequent analysis for UVPM and solanesol. This first filter was backed by a Teflon™ o-ring spacer, and a perforated Teflon™ sheet as a spacer. Another Teflon™ o-ring spacer was then used before a final 47 mm diameter glass fibre filter (Whatman, UK) impregnated with sodium bisulphate solution (4% w/v before drying), which was used to trap nicotine (Figure 2).

Room concentrations of UVPM and solanesol were measured by collection onto a 25 mm diameter Teflon™ filter (Millipore, UK) with nicotine collected separately on XAD-4 adsorption tubes (SKC, UK).

Chamber Monitoring

A number of methods were used to monitor the chamber atmospheres in real time, in addition to UVPM, solanesol and nicotine sampling. Carbon monoxide was measured with an electrochemical cell (Bedfont, UK); ETS particulate concentration was monitored with a MiniRam (calibrated for ETS measurement), and particle number was determined using

a condensation nucleus counter (CNC-TSI, USA). Further particle analysis and sizing were carried out using a Las-X laser spectrometer (Malvern, UK) based on light scattering and a quartz crystal microbalance (QCM - Berkeley Telonic, UK). Flow rates for all sampling equipment were measured before the start of each run using an bubble flow meter which electronically times the passage of a soap film across a fixed volume (Gilibrator, Gilian, USA).

ETS Particulate - Chamber

ETS particulates were collected in the $14m^3$-chamber during each volunteer exposure by sampling onto two 25 mm diameter Teflon™ filters at two measured flow rates of approximately 5 and 15 $l.min^{-1}$ respectively for the duration of the 60 minute volunteer exposure. The tar from each filter was dissolved in 5ml methanol and its UV absorbance measured at 325 nm. The ETS particulate concentration was then calculated from a calibration curve of UVPM (absorbance at 325nm) versus mass, from a calibration prepared from serial dilutions from ETS particulate samples of known dry mass.

ETS Particulate - Exhaled

Tar from the 47 mm diameter Teflon™ exhalate filter was dissolved in 5ml methanol and the UV absorbance measured at 325 nm. The exhaled particulate was then calculated from a calibration curve of UVPM (absorbance at 325nm) versus dry mass and corrected for dead space effects. Previous measurements had determined that the slope of the calibration curve for exhaled measurements was not significantly different for that obtained from ETS measurements in the chamber.

Solanesol

The methanol extracts used for UVPM measurements were also used for solanesol analysis by reverse phase HPLC (Waters Resolve C18 : 15cm, particle diameter 5 micron, pore diameter 90 Angstrom). The flow rate (100% methanol solvent) was 1 $ml.min^{-1}$, the injection volume 100 µl and solanesol was detected by UV absorption at 210 nm. Solanesol was quantified by reference to a prepared standard curve (Solanesol : Sigma S7854) over the range 0.125 to 1.0 $\mu g.ml^{-1}$. The limit of detection for solanesol was 0.05 $\mu g.ml^{-1}$, with a between-run precision of 6.9%. All samples were analysed in duplicate. It should be noted that a high degree of variability was seen in the HPLC traces for solanesol analysis, with differing degrees of complexity. This often required the solanesol concentration to be calculated from a sloping baseline or as a shoulder peak.

Nicotine - Chamber

Nicotine samples were collected from the chamber during the 60 minutes of each run using XAD-4 adsorption tubes (SKC, UK, (Hammond *et al*, 1987, Ogden, 1989)). Two samples were obtained, each with a measured sampling rate of approximately 4 $l.min^{-1}$. The tubes were eluted using 2 ml ethyl acetate containing 0.1% w/v triethylamine with 0.909 $\mu g.ml^{-1}$ quinoline as an internal standard. Samples were analysed on a HP 5890 GC with NP detection .. Two µl of sample were injected onto the column of RTX-5 (30m x 0.32 mm id with 1 µm film thickness).

Nicotine - Exhaled

The acidified, bisulphate-impregnated glass fibre filter was extracted with a mixture of 5M sodium hydroxide (20 ml) and di-isopropyl ether (50 ml). Quinoline (1.05 $\mu g.ml^{-1}$) was used as internal standard with subsequent GC/NPD analysis as above.

Volunteers

Twelve male and twelve female volunteers, all non-smokers, were recruited by advertisement from the workforce at the Harwell Laboratory, following approval of the study by the AEA Technology Ethical Committee. Each volunteer was interviewed and examined by a medical practitioner to determine fitness to participate, and each performed a vitalograph test to determine lung capacity. An informed consent for participation in the study was then obtained from each volunteer.

Each subject was given training on the valving apparatus in the absence of smoke in the chamber, for at least 15 minutes. On separate days, each male subject participated in a low- and a high-level exposure of 60 minutes; each female was subject to a high level exposure only. Subjects were exposed singly or in pairs within the chamber, where two sets of valving apparatus were available. All subjects were asked to minimise their ETS exposure prior to each experiment, although the workplaces of the majority of the subjects were non-smoking areas.

RESULTS

Chamber Monitoring

All measurement methods used demonstrated that following an equilibration period, steady-state concentrations of aged and diluted sidestream smoke could be maintained over periods of several hours. Figure 3 shows particle (CNC) and mass (MiniRam) concentrations measured during typical high and low level exposures. Volunteers exposures in each case took place during the period between 30 and 90 minutes as shown in Figure 3. A mass median diameter (MMD) of 0.14 µm ($\sigma_g = 2.6 : n = 5$) was measured using the quartz crystal microbalance (QCM), in effect a cascade impactor with cut-off stages of 0.1, 0.2, 0.4, 0.7, 1.1, 2.0, 3.6, 7.0, 12.0, and 24.8 µm. A count median diameter (CMD) of 0.125 µm ($\sigma_g = 1.5 : n = 20$) was measured using the Las-X spectrometer, with particle counts measured in the 0.11, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.65, 0.8, 1.0, 1.25, 1.5, 2.0, 2.5 and 3.0 µm cut-off regions.

Exposure Concentrations

Mean UVPM, solanesol and nicotine concentrations at the various exposure levels are shown in Table 1. The low level exposure is at levels higher than, but of the same order of median values reported from environmental surveys reviewed in the literature (e.g. Guerin *et al.* 1992). The higher level exposures were carried out such that concentrations of the exhaled components captured in the filter pack would be significantly greater than the detection limits for the appropriate analytical techniques, so that improved analytical precision would be achieved. The lower level measurements, at concentrations approaching analytical detection limits, could then be assessed critically.

Solanesol levels are approximately 1.3% of total particulate levels, consistent with literature data (Ogden and Maiolo, 1988). It was observed that there was a greater variation in nicotine concentrations than was seen for the corresponding ETS particulate and solanesol measurements. This was reflected in the particulate / nicotine ratio measured in the chamber (Table 1 and Discussion).

Details of the volunteers are listed in Table 2. There was no significant difference in age between the two groups, but the men were significantly taller and had significantly greater Forced Vital Capacities (FVC - a measure of lung volume). Both groups showed increased vital capacities over the expected norm for age and height, reflecting a 'healthy worker' effect in recruitment for the study.

Retention

A summary of retention of each measured component in the respiratory tract is shown in Table 3. The summary table lists Mean Retention ± Standard Deviation for the group. Predicted retention values for particulates using a model of respiratory particle deposition (LUDEP - Birchall, 1991) are also shown. Standard errors were approximately 5% for high level exposures and 10-15% for male, low level exposures arising principally from the component concentrations of the exhalate being close to the analytical detection limits.

The retention of ETS particulate (as UVPM) in the male volunteers is consistent at both high and low level exposures, with a mean retention fraction of 41 ± 14% for high level exposures (95% CI = 31 - 50%) and 36 ± 20% for low level exposures (95% CI = 20 - 52%). Retention in females assessed by UVPM was found to be significantly lower than

that for men with an average retention of 17 ± 10% (95% CI = 10 - 23%) at the higher level.

No solanesol results have been reported from the low level exposures for men and women, as exhaled solanesol levels were approaching the detection limit of the assay, yielding absolute errors of ± 15 - 60%. At the high exposure level, mean retention fractions of 40 ± 20% for men (95% CI = 30 - 50%) and 27 ± 14% for women (95% CI = 14 - 40%) were observed. Thus, solanesol retention for male volunteers is in agreement with the UVPM values. HPLC assays for solanesol from 4 exhaled filters from females (A, B, F and L) and 2 for male subjects (D and J) gave complex traces over high and rising baselines, in which it was difficult to determine peak areas accurately, in contrast to the majority of analyses where normal baselines were achieved. These outliers suggested solanesol retentions of << 0% or > 100%. These data were excluded from the summary table calculations (Table 3), being rejected on the grounds of unsatisfactory resolution of the HPLC traces.

Mean fractional nicotine retentions of 77 ± 17% and 71 ± 12% (95% CI = 62 - 88%) were seen for the male group of volunteers under the high and low exposure conditions respectively. Retention in the female group of volunteers was 81 ± 16% (95% CI = 70 - 91%) at the high exposure level.

Average breathing patterns for the volunteer groups (Table 4) show that there was consistency in breathing pattern for high and low level exposures. In general, men took larger breaths but fewer of them, reflecting their larger lung capacity. Both male and female volunteers, on average, used only 17% of their lung capacity during the breathing cycle of the experiment. This is consistent with breathing at rest, and implies that the subjects did not have to make efforts to exhale forcibly.

DISCUSSION

Male Volunteers

The results obtained for retention of ETS components in male volunteers show a consistent pattern. For particulates as UVPM, mean retention was 41 ± 14% and 36 ± 20% of that inhaled at the high and low exposure levels respectively, and this was confirmed by results for solanesol. These data are higher than previous assumptions made for particulate retention of 11 ± 4% (Mean ± SD) as reported by Hiller (1984). However, the deposition fractions reported by Hiller were for 0.41 µm mass median aerodynamic diameter (MMAD) smoke particles rather than the 0.14 µm MMAD particles reported in this study. Other recent studies in which ETS particle size has been determined in laboratory situations report ETS particle size at 0.15 - 0.25 µm MMAD at ETS concentrations typical of environmental exposure (Guerin *et al*, 1992). Thus lung deposition models would predict deposition fraction to be greater than those measured by Hiller as deposition by Brownian motion becomes more significant at the lower particle sizes (ICRP, 1966).

Model predictions of particle deposition were calculated to be 34 ± 8% applying each person's measured breathing pattern in the LUDEP lung model (Birchall *et al*, 1991), although no significant correlation was found for observed versus predicted values for UVPM or solanesol. Using the LUDEP model for the experimental conditions reported by Hiller, a deposition fraction of 22% was obtained. In each case, predicted extra-thoracic deposition is negligible, irrespective of mouth or nasal breathing, suggesting that total retention is approximately equivalent to lung deposition.

The experimental data are consistent with those from another study (Strong *et al*, 1993) in which total and regional deposition of ETS were measured using exhale capture

techniques plus a radiotracer (^{212}Pb) attached to the involatile particulate, allowing whole body monitoring for retention and excretion measurements. Total retention was 43 ± 17% (Mean ± SD) for a group of nine male volunteers following a fixed mouth breathing pattern of 6 x 1.0 litre breaths per minute, which was very close to the mean pattern seen for the male volunteers in this study. The inter-subject variation observed in this study was similar to that reported by Strong *et al*, 1993 and Hiller, 1984, although greater than predicted by the model (Birchall *et al*, 1991).

Particulate retention data for female volunteers are less clear-cut than the data for the males. Retention fractions for solanesol and UVPM were approximately 27 ± 14 % and 17 ± 10% respectively. The LUDEP particle deposition model with measured breathing patterns predicted particle deposition of 26 ± 5% (Birchall *et al*, 1991) and a significant correlation was observed for observed particulate deposition (UVPM) versus predicted (LUDEP) values ($p<0.001$). Inter-subject variation was similar to that observed for male subjects. Although nicotine retention data for the female group were satisfactory, only 67% of the female volunteers gave reproducible results for solanesol. It was initially assumed that solanesol data exhibiting a retention of less than 0% were a consequence of problems in exhalation and mask fitting; while this is possible in some cases, such an effect would not explain why the majority of the nicotine retention results for the group were reproducible. Both men and women were breathing to only 17% of their vital capacity during the experiments, suggesting that no discomfort was experienced. Analytical blanks for all analytes were satisfactory for chamber and exhalate generated samples, although complex baselines on a number of solanesol HPLC assays made interpretation difficult. Hence summary data in Table 3 excludes the outliers where 0% > x > 100%.

It is possible that the problems encountered with the assays for solanesol for female exhalate may arise from a gender difference. Similar anomalies were observed for UVPM

measurements in preliminary exposures for women with deposition values << 0%. Generally, women have a higher proportion of adipose tissue in body composition, and that this may act as a depot for organic molecules. Thus, release of these components may differ from men and account for measurement differences in the presence of ETS, as blanks were unaffected.

Although mean ETS particulate retention for each gender is greater than the value of 11% reported by Hiller, 1984, it should be noted that total intake of ETS particulate remains low. Many authors have used the concept of 'cigarette equivalents' in order to give a measure of dose relative to the dose from a single cigarette in an active smoker. By assuming toxicological equivalence of the two smokes, risk estimates have been constructed. Estimates of 'cigarette equivalents' have varied from 0.024 to 27 cigarette equivalents per day, a range which has been seen as illogical (US Department of Health and Human Services, 1986). Calculations for the highest estimate in this report divided the calculated ETS particulate intake by a mainstream smoke intake of 0.55 mg per cigarette, a minimum yield value for cigarette brands available in the US. However, the original authors of the cited work quote a sales weighted average mainstream smoke yield of 17 mg per cigarette (Repace and Lowrey, 1980). Use of this more realistic value would reduce the range quoted in the US Surgeon General Report to 0.024 to 0.9 cigarette equivalents per day. While early estimates based ETS dose on ETS concentration per unit volume multiplied by volume inhaled, more recent calculations of ETS dose have taken into account deposition fraction and adjusted exposure via time-activity matrices which estimate the duration of exposure, at work and/or home, with ventilation volumes adjusted for activity such as light work or exercise (Holcomb, 1993). On this basis, Holcomb has calculated daily ETS particulate intake under a variety of lifestyles, with maximum intakes of 109 $\mu g.day^{-1}$ for men and 58 $\mu g.day^{-1}$ for women, and 45 and 34 $\mu g.day^{-1}$ respectively for home and leisure exposure only. However, exposure concentrations were based on

attributing specific ETS exposure to the difference in particulate concentration between smoking and non-smoking areas. The values obtained are in contrast to mean and maximum ETS particulate intakes of 1430 and 14 300 $\mu g.day^{-1}$ respectively, which represent the maximum reported literature values as calculated by Repace and Lowrey, 1980.

ETS particulate intake has been calculated for home and workplace using the deposition and ventilation data measured in this study with with exposure concentrations based on those suggested by Holcomb (1993). Summarising likely exposure, Holcomb uses a series of studies carried out in the US and Canada, suggesting that the difference in mean RSP or UVPM measurements for smoking (S) and non-smoking (NS) areas were 27 $\mu g.m^{-3}$ for homes (50S v 23NS), 22 $\mu g.m^{-3}$ for offices and public places (68S v 46NS), 42 $\mu g.m^{-3}$ for restaurants (132S v 90NS), 30 $\mu g.m^{-3}$ for trains (216S v 186NS) and 104 $\mu g.m^{-3}$ for bars and taverns (S data only), and that these differences relate to exposure attributable to ETS. Our calculations have been carried out using both the absolute mean particle concentrations in smoking areas (S) and the difference between smoking and non-smoking areas (S-NS), which Holcomb attributes to the ETS component of the ambient particulate. Thus, absolute (S) values will give a maximum value for intake from total particulate exposure.

In our calculation, a 16 hour exposure at the ventilation rates measured during the study is assumed (0.42 m^3 per hour for men and 0.36 m^3 per hour for women). Workplace exposure assumes an 8 hour exposure at twice the resting ventilation rate. Fractional deposition values were 0.41 for men and 0.27 for women, and the calculated values are shown in Table 5. Values are higher than those reported by Holcomb (1993) reflecting the increased deposition fraction used in our study, although measured ventilation rates in our work were lower than the equivalent values used by Holcomb.

It has been reported that the fractional deposition of mainstream tobacco smoke is greater than for ETS with values ranging from 47 - 90% (US Surgeon General, 1986) with most reported values at the higher end of this range. Measurements of tar deposition in mainstream cigarette smokers using radiotracers have shown an intake of 453 mg per day (range = 307 - 728 mg per day) in male middle-tar smokers with an average intake of 13.5 mg per cigarette (range = 7.4 - 22.0 mg per cigarette) (Pritchard *et al*, 1988a, McAughey *et al*, 1989). Equivalent data for female smokers give values of 284 mg per day (range = 111 - 488 mg per day) and 12.5 mg per cigarette (range = 9.3 - 21.2 mg per cigarette) (McAughey *et al*, 1991). In each case, the mean delivery of the cigarette was equivalent to the Sales Weighted Average Tar for the UK, consistent with the measured yield of the experimental cigarette. Cigarette equivalent data and daily intake as a fraction of mainstream intake are shown in Table 6, confirming that average doses from ETS are small.

Although the nature of this study was such that regional deposition could not be measured, regional deposition data (Strong *et al*, 1993) for ETS particulate using a radiotracer suggests that the regional deposition patterns of mainstream tobacco smoke and ETS particulate are different, with ETS particulate depositing more deeply in the lung. Therefore, direct comparisons of particulate retention on a cigarette equivalent basis may be inappropriate. This concern is supported by the US Environmental Protection Agency, who chose not to consider the use of 'cigarette equivalents' in their recent publication reviewing the respiratory health effects of passive smoking (USEPA, 1992) for a variety of reasons. For example, although mainstream tobacco smoke (MS) and ETS are qualitatively similar with respect to chemical composition, the absolute and proportional quantities of the smoke components, their physical state and their partitioning between phases can differ. Further differences included variations in particle size between MS and

ETS and different breathing patterns in smokers and non-smokers, leading to differences in the distribution and deposition of each type of smoke in the respective populations. Subsequent metabolic differences were also discussed, suggesting dose-response associations were likely to be non-linear.

Nicotine retention in men and women was significantly different from particulate retention, with observed mean retention values of 70 to 80%. This is consistent with data from a study by Iwase *et al* (1991) which measured retentions of nicotine of 60 to 80% (mean ± SD: 71.3 ± 10.2%) in a group of 17 non-smoking women inhaling sidestream smoke. Data were obtained by a similar inhaled minus exhaled technique to that reported here, but using nicotine denuders rather than a bisulphate impregnated filter.

However, these data confirm that nicotine retention is not a representative marker of ETS exposure. This is particularly important with respect to a recent publication (Nelson *et al*, 1992) which suggests that the ratio of nicotine to both vapour- and particulate-phase components is highly variable with respect to sampling time and ventilation. Thus, environmental surveys of nicotine exposure will be of greater variability than our data and those of Iwase *et al* (1991), which also represent a steady state situation.

The ratio of airborne particulate to nicotine in the exposure chamber was significantly different at the two exposure levels in this study (Table 1). This is consisent with previous data in which the ratio of particulate nicotine / vapour nicotine increased with particulate concentration (Guerin *et al*, 1992). This pattern is consistent with a dynamic reversible equilibrium of nicotine between vapour and particulate phases. In this study, and in most environmental studies, only nicotine vapour was measured.

These data support the conclusion of Nelson *et al*, 1992, suggesting that nicotine intake cannot be related directly to particulate intake. The variability of particle / vapour ratio precludes calculations of particle retention when only environmental nicotine concentrations are known, or when nicotine exposure has been extrapolated from bioassay data for nicotine or cotinine (a major nicotine metabolite).

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Table 1 - ETS exposure levels

Experiment	UVPM Mean ± SD ($\mu g.m^{-3}$)	Solanesol Mean ± SD ($\mu g.m^{-3}$)	Nicotine Mean ± SD ($\mu g.m^{-3}$)	UVPM/Nicotine Ratio Mean ± SD
Male, High level (n=12)	920 ± 120	11.7 ± 1.5	29 ± 13	31.7
Male, Low level (n=10)	150 ± 60	1.5 ± 0.7	9.5 ± 2.3	15.8
Female, High level (n=12)	1040 + 140	13.2 ± 3.2	35 ± 10	29.7

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Table 2 - Volunteer data

Subject	Male (n=12) Mean ± SD (Range)	Female (n=12) Mean ± SD (Range)
Age (y)	33 ± 5 (26 - 45)	33 ± 10 (22 - 51)
Height (m)	1.76 ± 0.05 (1.68 - 1.85)	1.65 ± 0.06 (1.57 - 1.77)
FVC (l)	5.82 ± 0.74 (4.78 - 7.29)	3.95 ± 0.72 (2.82 - 5.20)
FVC/Norm (%)	118 ± 18 (98 - 160)	112 ± 17 (86 - 136)

Table 3 - ETS Retention

Condition (No. of subjects)	Male Retention ± SD (%) (Range)	Female Retention ± SD (%) (Range)
UVPM High (12M, 12F)	41 ± 14 (12 - 65)	17 ± 10 (3 - 39)
UVPM Low (10M)[a]	36 ± 20 (5 - 65)	-
Solanesol High (10M, 8F)[b]	40 ± 20 (20 - 57)	27 ± 14 (10 - 58)
Nicotine High (12M, 12F)	77 ± 17 (41 - 100)	-
Nicotine Low (10M)[a]	71 ± 12 (49 - 88)	81 ± 16 (39 - 96)
Predicted particulate retention (LUDEP)	34 ± 8 (28 - 50)	26 ± 5 (20 - 39)

[a] 2 male subjects declined to undertake low level exposure

[b] excluding values of << 0% or > 100%

Table 4 - Breathing Patterns

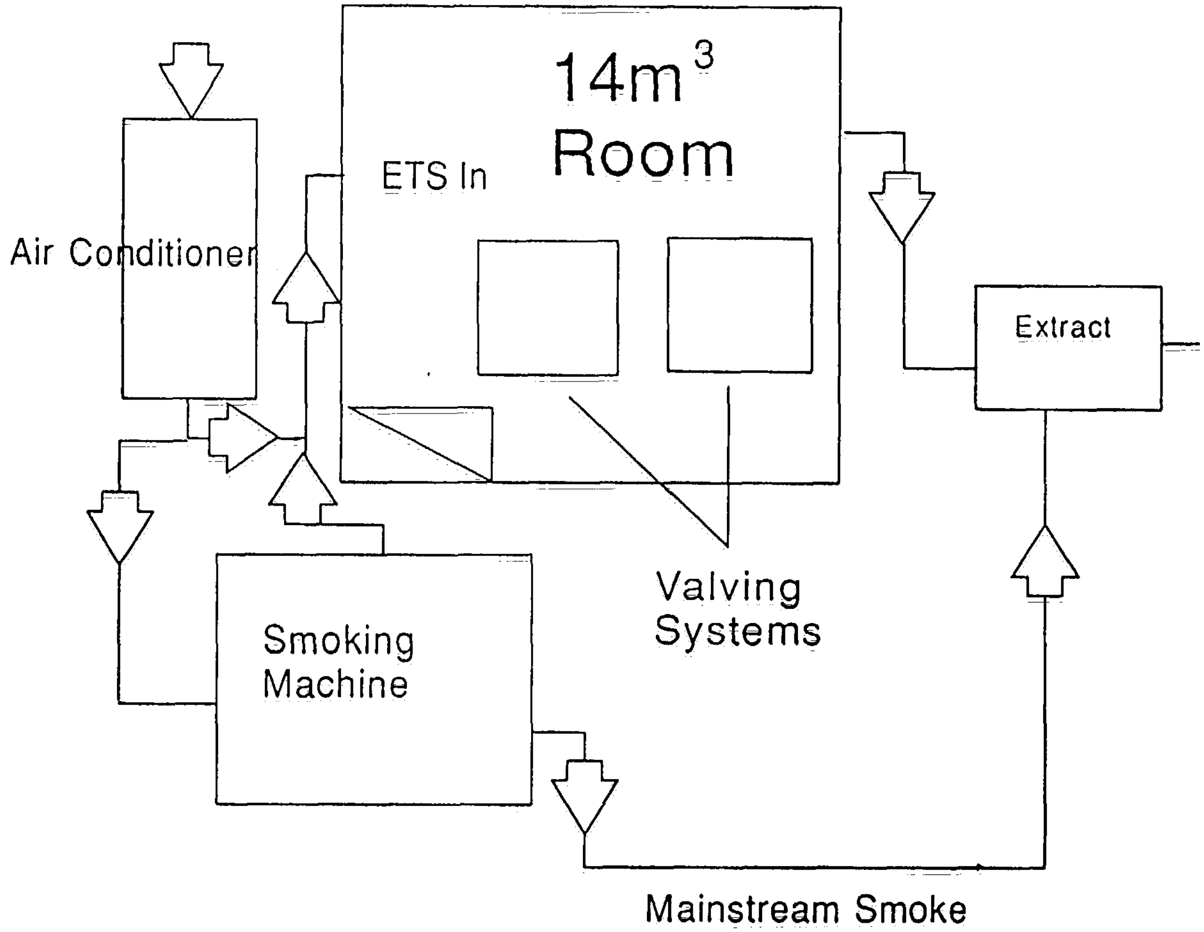
Condition	Male, High Level Mean ± SD (Range)	Male, Low Level Mean ± SD (Range)	Male, High Level Mean ± SD (Range)
Volume (l)	439 ± 119 (226 - 647)	422 ± 86 (326 - 629)	358 ± 122 (242 - 606)
Number of Breaths	490 ± 170 (187 - 766)	450 ± 133 (254 - 726)	566 ± 154 (307 - 802)
Volume per Breath (l)	0.98 ± 0.33 (0.46 - 1.74)	1.02 ± 0.31 (0.48 - 1.53)	0.66 ± 0.21 (0.40 - 1.02)
%FVC per Breath	17 ± 8 (8 - 36)	17 ± 6 (8 - 32)	17 ± 5 (10 - 27)

Table 5 - Estimated ETS / Particulate Intake

Condition	Particle Concentration ($\mu g.m^{-3}$)	Exposure Duration (h)	Ventilation ($m^{-3}.h^{-1}$)	Deposition Fraction	Particulate Intake ($\mu g.day^{-1}$)
Home					
Male (S)	49	16	0.42	0.41	135
Male (S-NS)	27	16	0.42	0.41	74
Female (S)	49	16	0.36	0.27	76
Female (S-NS)	27	16	0.36	0.27	42
Work					
Male (S)	68	8	0.84	0.41	187
Male (S-NS)	22	8	0.84	0.41	61
Female (S)	68	8	0.72	0.27	106
Female (S-NS)	22	8	0.72	0.27	34

Table 6 - Comparison of particulate intake from cigarette smoking and exposure to ETS

Exposure Condition	Particulate Intake ($\mu g.day^{-1}$)	Daily intake as % smoker intake	Daily Cigarette Equivalent
Home			
Male (S)	135	0.030	0.010
Male (S-NS)	74	0.016	0.005
Female (S)	76	0.027	0.006
Female (S-NS)	42	0.015	0.003
Workplace			
Male (S)	187	0.041	0.014
Male (S-NS)	61	0.013	0.005
Female (S)	106	0.037	0.008
Female (S-NS)	34	0.012	0.003


14m³
Room
ETS In
Air Conditioner
Extract
Smoking
Machine
Valving
Systems
Mainstream Smoke

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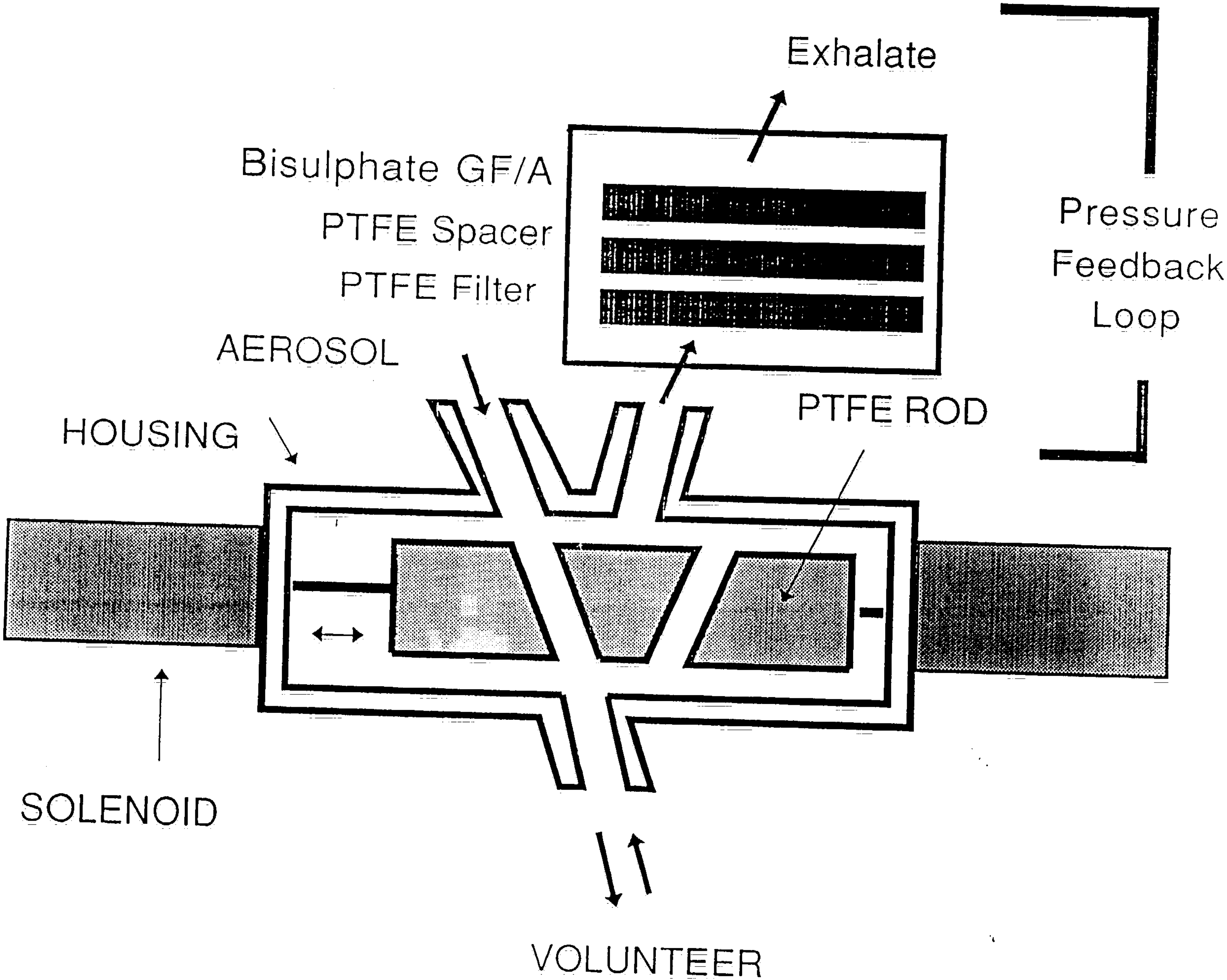
Exhalate
Bisulphate GF/A
PTFE Spacer
PTFE Filter
Pressure
Feedback
Loop
AEROSOL
HOUSING
PTFE ROD
SOLENOID
VOLUNTEER

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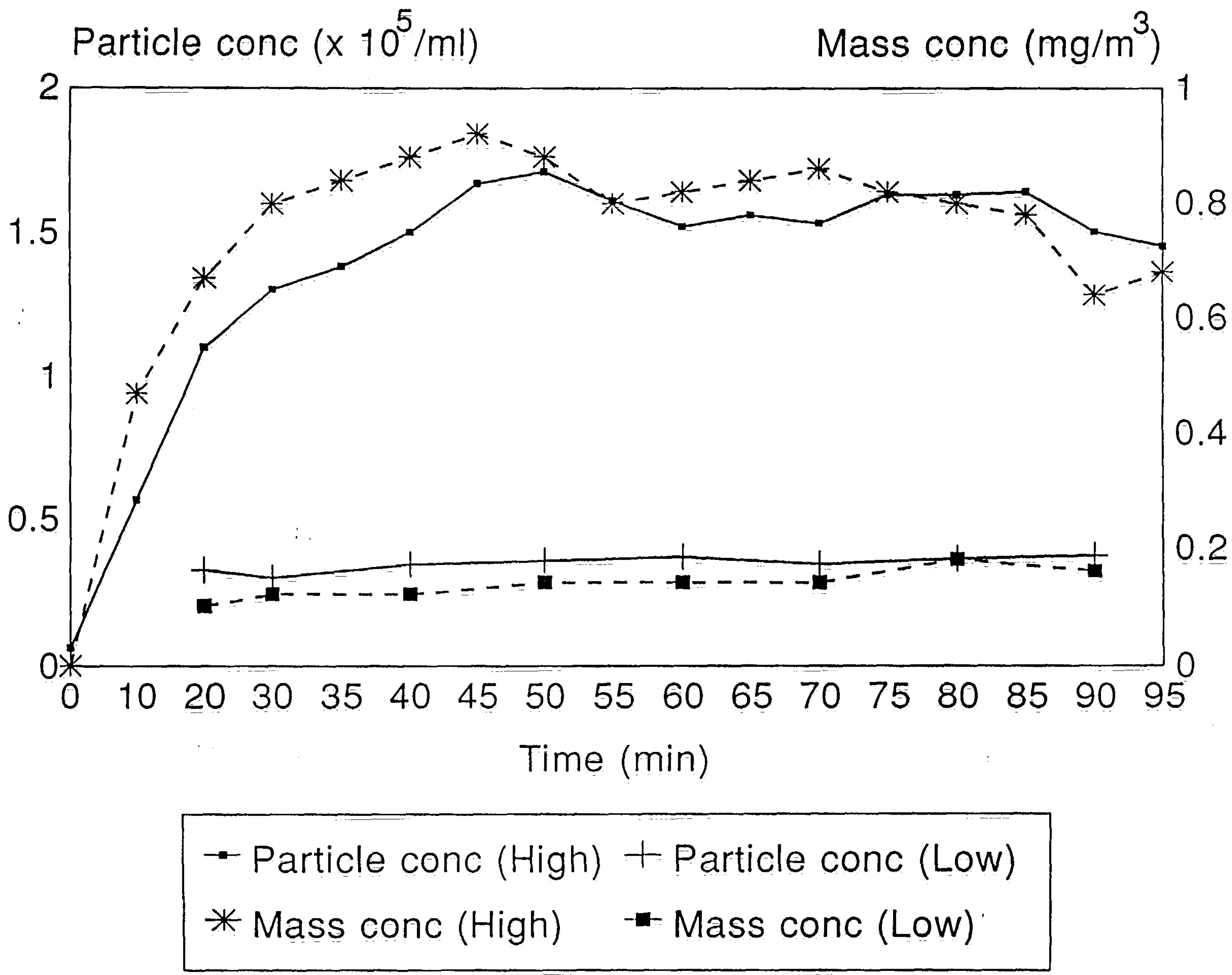

Particle conc (x 10^5/ml)
Mass conc (mg/m^3)
2
1.5
1
0.5
0
1
0.8
0.6
0.4
0.2
0
0 10 20 30 35 40 45 50 55 60 65 70 75 80 85 90 95
Time (min)
Particle conc (High)
Particle conc (Low)
Mass conc (High)
Mass conc (Low)

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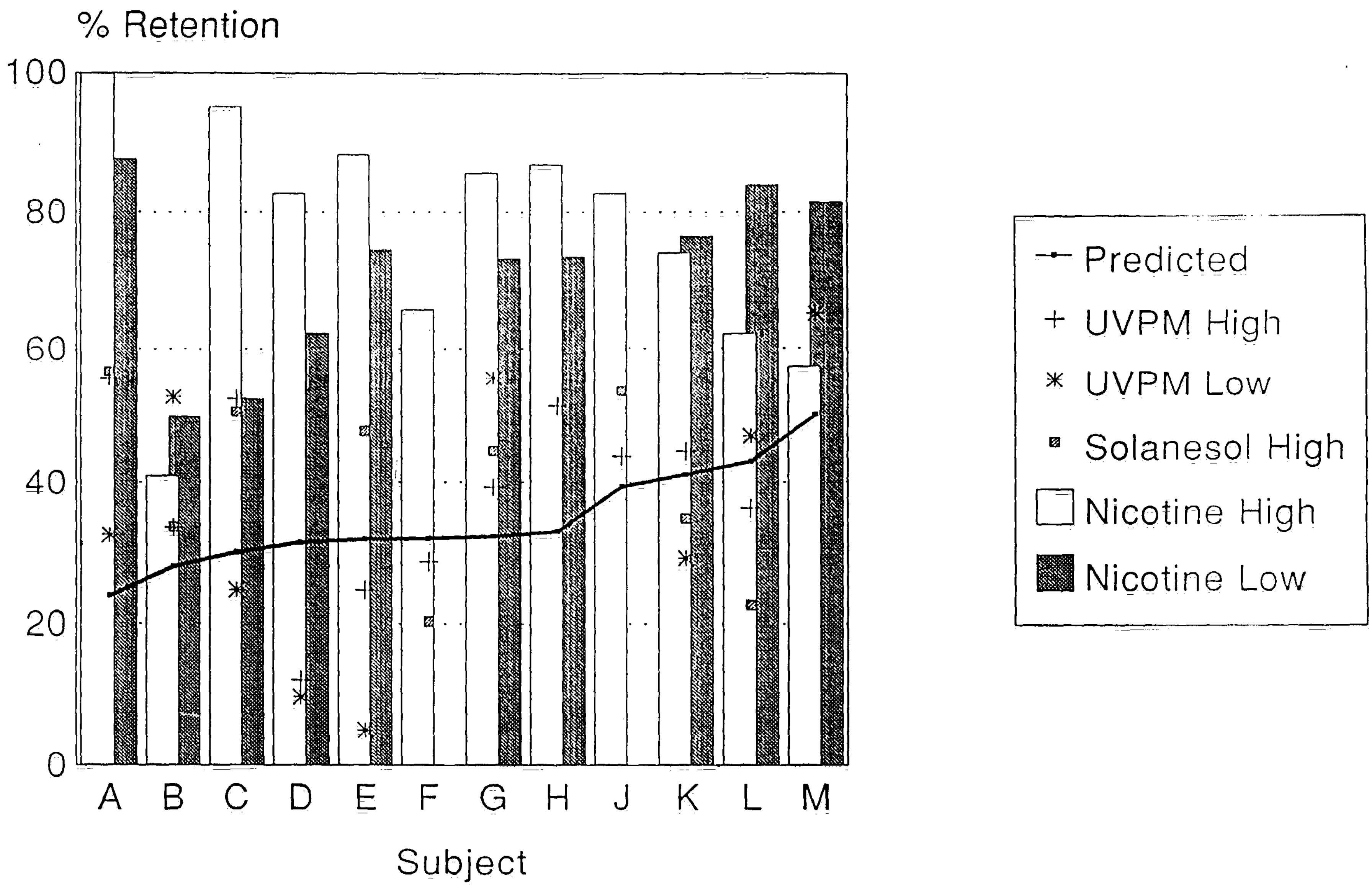

% Retention
100
80
60
40
20
0
A
B
C
D
E
F
G
H
J
K
L
M
Subject
Predicted
UVPM High
UVPM Low
Solanesol High
Nicotine High
Nicotine Low

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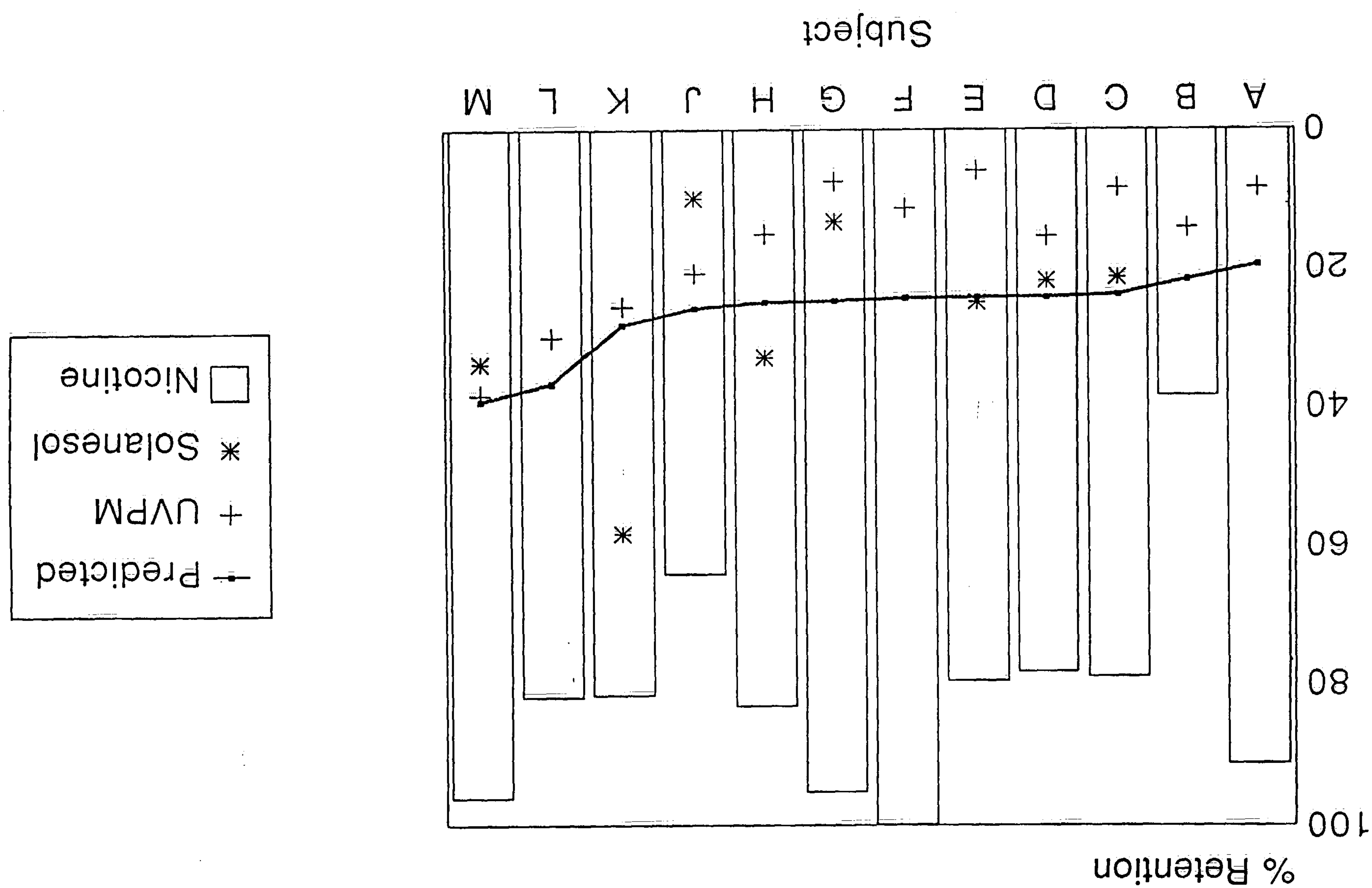

% Retention
0
20
40
60
80
100
A
B
C
D
E
F
G
H
J
K
L
M
Subject
Predicted
UVPM
Solanesol
Nicotine

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^{32}P-postlabelling analysis of DNA adducts in monocytes of smokers and passive smokers

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Summary. In a controlled study, ten male volunteers were subjected to different smoking and passive smoking conditions. After 60 h of strictly controlled nonsmoking, five smokers were exposed to mainstream smoke only, while five nonsmokers were exposed to the gas phase of environmental tobacco smoke (ETS). In a second experiment smokers were mainstream and ETS exposed, while nonsmokers were exposed to complete ETS. Blood was drawn before and after smoking and DNA adducts were analysed from blood monocytes by the ^{32}P-postlabelling assay, using the nuclease P1 enhancement method. We detected DNA adducts in monocytes of all probands. These adducts unrelated to smoking showed interindividual differences but only minor intraindividual changes in four samples of the same donor. After smoking interindividually variable additional adducts were visible in active smokers only. These smoking-related adducts had disappeared after 40 h of nonsmoking and reappeared again in three out of five smokers after the second smoking period. We conclude that smoking causes an interindividually variable pattern of DNA adducts in active smokers. These adducts disappear in less than 2 d owing to the fast turnover of monocytes in the intravascular system. The effects described could not be observed in heavily exposed passive smokers.

Key words: Postlabelling assay – Smoking – Passive smoking – Monocytes

Introduction

Although a series of suspected carcinogenic substances have been found in cigarette smoke, there is still no definite answer as to which specific compounds are responsible for the increased risk of smokers in acquiring lung cancer [6]. In addition, there is a growing need to evaluate a possible carcinogenic risk in passive smokers, suggested by several epidemiological studies, although the extent of the effect remains to be investigated [16]. Since the covalent binding of xenobiotics or their metabolites to DNA is an important indicator for genotoxic stress [15], we studied the formation of DNA adducts in monocytes of smokers and passive smokers. The ^{32}P-postlabelling assay, being one of the most sensitive methods, seems to be especially suited for analysing the formation of DNA adducts resulting from the exposure to complex mixtures of unknown composition [17]. After pretreatment with nuclease P1 the nucleotides carrying a covalently bound adduct are selectively labelled with ^{32}P-phosphate. This allows the detection of PAH or bulky adducts with a sensitivity of a few molecules per genome [5]. Though several studies using the ^{32}P-postlabelling assay have shown a higher level of adducts in several tissues of smokers as compared to controls [12, 14], this has not yet been possible with human white blood cells [10, 11]. Here we used isolated monocytes, because these cells are metabolically active without pretreatment [1] and are therefore expected to reflect the formation of DNA adducts in vivo.

Materials and methods

Design of the study. Ten male healthy volunteers, five smokers (age 19–28) and five nonsmokers (age 23–29) refrained from smoking 60 h before the first blood sample was drawn. During the 6 d of the study the probands received a standardized diet, equal in amount and quality. Two different exposure conditions were applied: The third day smokers were exposed to mainstream smoke only and the fifth day to mainstream smoke and ETS; nonsmokers were exposed to the gas phase of ETS for 8 h on the third day and to complete ETS for 8 h on the fifth day. In the first experiment the five smokers had to smoke a total of 24 cigarettes (1 cig./20 min) and exhaled the smoke through an oneway-valve into the exposure

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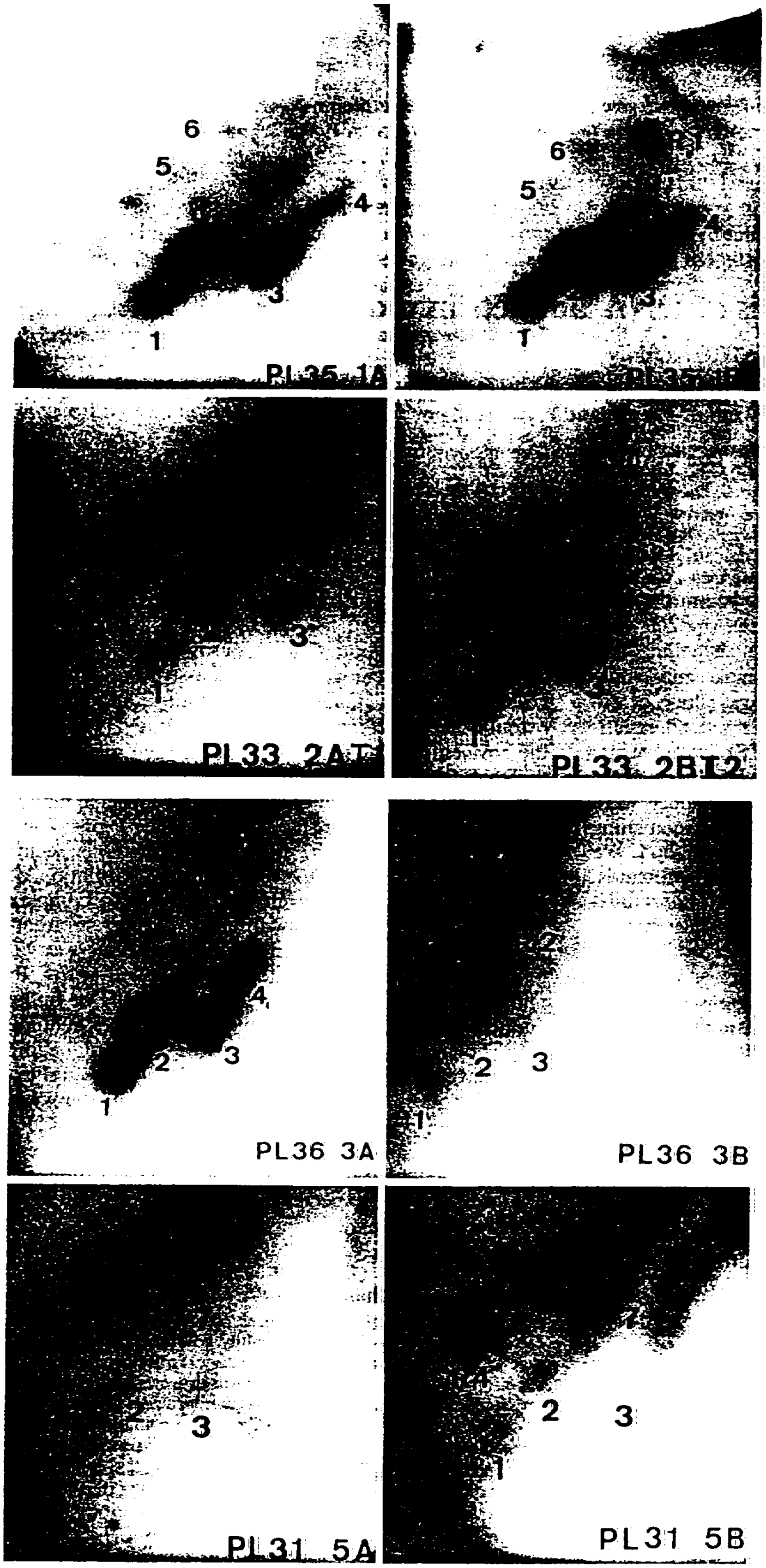

Fig. 1. Autoradiographs of PEI-cellulose t.l.c. maps of ^{32}P-labelled digest of DNA from peripheral blood monocytes of smokers (probands 1, 2, 3, 5). Sample A taken before and sample B taken after the first smoking period. R1, R2, R4, R5 are additional spots detected after smoking. Screen intensified autoradiography at −80°C for 72 h

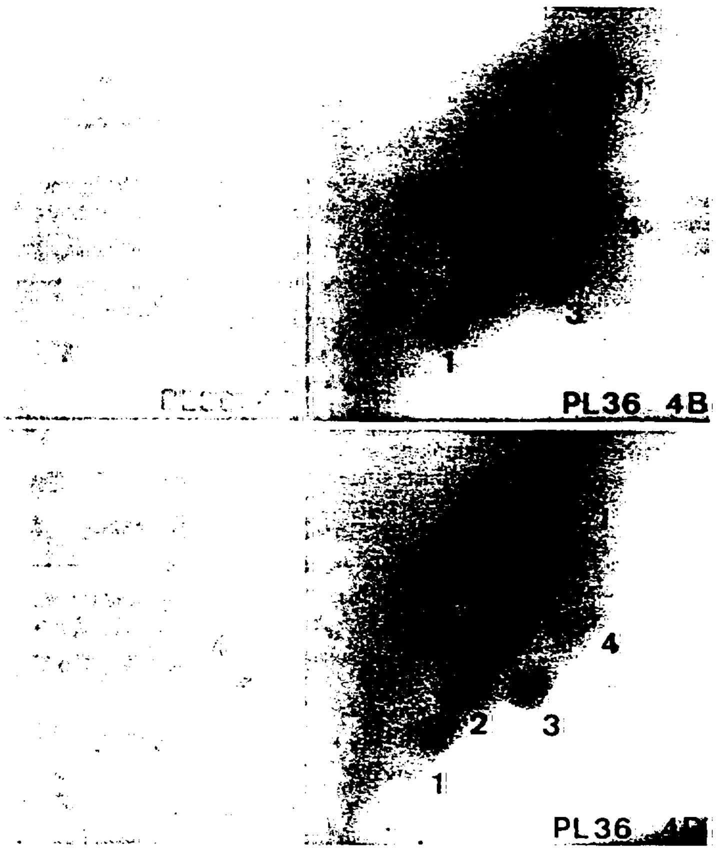

Fig. 2. Autoradiographs of PEI-cellulose t.l.c. maps of ^{32}P-labelled digest of DNA from peripheral blood monocytes of a smoker (proband 4). Samples taken before and after the first (A, B) and the second (C, D) smoking period. Smoking related adducts are only visible after smoking (R1, R2)

... tobacco smoke ... room had to carry ... and limited the ... sample A) and after ... were taken. After ... equal conditions ... exposed to the en- ... taken previous to ... ample D). Room air ... days and amounted ... nicotine and 3 to

... Blood (60 ml) was ... syringe by venous ... fully layered over ... en) in sterile 10-ml ... g for 40 min. The ... ere collected and ... Gibco, Grand Is- ... calf serum (FCS, ... g/l sodium bicar- ... erred into culture ... d at 37°C for 24 h ... here to allow the ... Floating lympho- ... er with the culture ... th isotonic saline, ... and stored frozen.

... sion was placed on ... µm) and the cells were lysed for 1 h at 37°C by an aqueous solution of sodium dodecyl sulfate (SDS, 20 mg/ml, Merck, Darmstadt, FRG), proteinase K (0.5 mg/ml, Merck) 0.05 M sodium phosphate and 0.05 M potassium phosphate. Subsequently the filters were washed with a 0.02 M Na_2EDTA solution, treated for 15 min with RNAse A (0.15 mg/ml, Boehringer Mannheim), and the remaining DNA was washed with a solution of 5 mM sodium phosphate and 5 mM potassium phosphate. Then the filters were cut into small pieces and DNA was dissolved in 2 ml H_2O by a short ultrasonic treatment. The amount of DNA was determined after incubation of an aliquot of the solution with diaminobenzoic acid and subsequent fluorescence spectrometry with an excitation wavelength of 408 nm and emission wavelength of 500 nm [2]. Calf-thymus DNA (Boehringer, Mannheim, FRG) was used as a standard.

^{32}P-postlabelling. DNA samples (2 to 8 µg DNA) were evaporated to dryness and digested overnight at 37°C in a total volume of 4.8 µl containing 0.125 U micrococcal nuclease (Sigma N-3758, Deisenhofen, FRG), 0.4 mU spleen phosphodiesterase (Boehringer, Mannheim, FRG), 17 mM sodium succinate and 8 mM CaCl. The digest was then treated with 4.8 µl of an aqueous solution of nuclease P1 (1 U, Sigma N-8630), sodium acetate (0.12 M) and $ZnCl_2$ (0.09 mM). The samples were incubated at 37°C for 1 h and the reaction was terminated by adding an aqueous solution (1.9 µl) of 0.5 M Tris base [11]. The DNA samples (5 µl) were then ^{32}P-labelled with 5 µl of a solution prepared by mixing 25 µl [Gamma-^{32}P]ATP (250 µCi, >5000 Ci/mmol, Amersham International), 7.5 µl T4 polynucleotide kinase 3'phosphatase free (67.5 U, Boehringer, Mannheim, FRG), 13.4 µl H_2O and 25 µl of buffer (200 mM Bicine, 100 mM $MgCl_2$, 100 mM dithiothreitol, 10 mM spermidine). After an incubation for 30 min at 37°C, potatoe apyrase (60 mU, Sigma A-6132) was added to terminate the reaction. The labelled nucleotides were

resolved by 4-dimensional chromatography on PEI-cellulose t.l.c. sheets (Macherey-Nagel Duren, FRG). The plates were first developed with 1 *M* sodium phosphate, pH 6.0 to wash polar adduct-free nucleotides on to a paper wick. The paper wick was removed and the plates were developed again in 3.5 *M* lithium formate, 8.5 *M* urea, pH 3.5, and after turning the plates 90° in 0.8 *M* lithium chloride, 0.5 *M* Tris-HCl and 8.5 *M* urea, pH 8.0. Subsequently the plates were developed in 1.7 *M* sodium phosphate on to a paper wick again to lower the background activity on the plates. The adducts were detected by screen intensified autoradiography for 72 h at −80°C (Xomat 5, Kodak, Rochester, NY, USA).

Results

Tobacco smoke exposure of the subjects is clearly reflected in significant increases in carboxyhemoglobin (COHb) after the 8-h exposure sessions. On Day 3 of the study, COHb increased by 7.1% in the active smokers and by 2.2% in the passive smokers. The corresponding increases for Day 5 were 7.7 and 2.6%.

The cells of all probands showed various DNA adducts which exhibited interindividual differences in amount and chromatographic behavior but were unrelated to smoking (sample A). After the first 8-h period of smoking additional DNA adducts appeared in samples of all smokers (sample B). These smoking related adducts varied individually (Fig. 1). In monocytes from sample C, (i.e. after a 40-h nonsmoking period), these additional adducts had disappeared, while the nonsmoking related adducts remained. After the second 8-h smoking experiment the additional adducts were detected again in samples of three smokers (Fig. 2). Nonsmokers exposed to ETS (passive smokers) did not show any additional adducts. There was no difference between samples taken after exposure to the gasphase only (sample B) and taken after exposure to total ETS (sample D).

Discussion

Smoking caused DNA adducts in peripheral blood monocytes of active smokers. Passive smoking did not lead to similar adducts. Since the samples were analysed using the nuclease P1 enhancement method it can not be concluded that passive smoking did not cause any DNA adducts at all. Nuclease P1 also dephosphorilates nucleotides carrying smaller and rather polar adducts, which for instance may arise due to tobacco specific nitrosamines. Therefore by the method used here, predominantly PAH derived or other bulky adducts are likely to be detected. Smoking-related DNA adducts in monocytes had already disappeared after a 40-h nonsmoking period. This is probably due to the relative short lifespan of blood monocytes ($t_{1/2} = 8$ h) [7] in the intravascular system and might explain why other authors [10, 11] were not able to detect differences between smokers and nonsmokers on peripheral blood leucocytes. Studies on lung tissue [12] and mouse skin [13] have shown a rather long half life of bulky DNA adducts, so that is unlikely to attribute the observed rapid disapearence of smoking-related adducts to a fast reacting repair mechanism in human monocytes.

All samples exhibited adducts not related to smoking (adducts 4–7). Similar adducts were not observed in fibroblast DNA, probably because these cells are cultivated under standardized conditions, and because preexisting adducts were diluted by multiple cell proliferation. Monocytes seem to mirror the actual amount of genotoxic stress and showed DNA adducts level in almost all samples analysed in our laboratory. The adducts 1, 2 and 3 (Fig. 1), however, which were found in almost all samples analysed in this study could also be found in fibroblast DNA, while calf thymus DNA never showed these spots. Therefore we assume that culture conditions may have caused these adducts. In addition, since their provenance remains finally unexplained, we do not make any attempt here to speculate about variable intensities of these spots. Other adducts in monocytes unrelated to smoking, however, must be either rather fast developing adducts since monocytes display a fast turnover in blood, or may represent pre-existing adduct levels in monocyte stemcells. Both, smoking-related adducts and unrelated adducts exhibited intraindividual variations. This could be explained by genetically determined differences in the amounts of various enzymes being responsible for metabolic activation or detoxification of exogenous and endogenous substances in the cell [3, 4, 8, 9].

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Cytogenetic effects of tobacco smoke exposure among involuntary smokers

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Summary

Tobacco smoke is highly genotoxic and produces chromosomal damage in several experimental systems. Active smokers have been shown to have an increased prevalence of somatic chromosome damage in their peripheral blood lymphocytes: this is seen in most cases as an increased sister-chromatid exchange (SCE) frequency and often also as increased structural chromosome aberrations (CAs). Among passive smokers, in association with exposure to environmental tobacco smoke, no such induction of chromosomal damage has been documented. In the present paper we report negative results on induction of chromosomal damage in 2 separate groups of intensive involuntary exposure to tobacco smoke, non-smoking restaurant personnel and newborn children of smoking mothers. While significant exposure in these groups is clearly seen in biochemical intake markers, e.g. cotinine and thiocyanate values in plasma, the conventional cytogenetic parameters, structural chromosome aberrations and sister-chromatid exchanges, are unable to detect the low exposures of involuntary smokers.

Most involuntary smoking occurs through exposure to environmental tobacco smoke (ETS), i.e., passive smoking. The chemical and biological characteristics of ETS have been discussed in this volume by Löfroth, Claxton and collaborators. Transplacental exposure of the unborn child is also, in fact, involuntary even if the chemical composition of the exposing agent, tobacco smoke, is different from ETS; it mainly consists of the transplacental components of main-stream smoke inhaled by the actively smoking mother.

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The well-documented genotoxic character of tobacco smoke condensates and all forms of tobacco smoke, main-stream, side-stream and environmental tobacco smoke in experimental conditions (see e.g., IARC, 1986; Claxton et al., this volume), also makes the exposure measures that are specific to the genotoxic character applicable in the natural exposure situation. In this report, we especially discuss the use of cytogenetic exposure measures, i.e., structural chromosome aberrations (CAs) and sister-chromatid exchanges (SCEs) in peripheral blood lymphocytes, to detect involuntary exposure to tobacco smoke in heavily exposed passive smokers and in newborn babies of smoking mothers.

Subjects and methods

The occupationally exposed group comprised 16 non-smoking waiters and 9 smoking waiters working in a night restaurant where there was no restriction in smoking. As controls, 7 non-smoking persons without obvious recent or past exposure to environmental tobacco smoke were included. A detailed description of the work-site restaurant (restaurant B) of the subjects was given in an earlier publication (Husgafvel-Pursiainen et al., 1986). The non-cytogenetic exposure parameters of the subjects (together with personnel from 2 other restaurants) have been described (e.g., cotinine, thiocyanate, carboxyhaemoglobin) in Husgafvel-Pursiainen et al. (1987) and the detailed results of the sister-chromatid exchange analysis by Husgafvel-Pursiainen (1987).

The present paper reports the analysis of chromosome aberrations in these same subjects and discusses the individual concordance of the 2 cytogenetic parameters, CAs and SCEs.

For the CA analysis the whole-blood microculture method was used with a 50-h culture time (see Mäki-Paakkanen et al., 1980 for details). The scoring was performed on coded slides by an experienced analyst counting 100 metaphases per subject.

The SCE frequencies of the transplacentally exposed group were studied from cord-blood samples taken at delivery. To avoid methodological variation between culture periods, a large batch of culture medium, including bromodeoxyuridine (20 μM; Calbiochem), phytohemagglutinin (1%; Wellcome), penicillin (100 units/ml) and streptomycin (100 μg/ml) in RPMI 1640 (Gibco) supplemented with 15% fetal calf serum (Gibco) was prepared for the whole study and stored at $-20°$C. The sister-chromatid exchange analyses were performed on duplicate cultures harvested and stained according to standard procedures (see Husgafvel-Pursiainen, 1987), scoring on codes, 50 second metaphases per individual.

The mothers were interviewed for their smoking habits and possible passive exposure a few days after the delivery. The group consisted of 17 actively smoking mothers and their newborn babies and 25 non-smoking mothers and newborns; only 7 of the non-smoking mothers described some exposure to environmental tobacco smoke. The biochemical intake markers measured from both the maternal and the cord-blood plasma samples were cotinine and thiocyanate; the analytical results and their discussion are reported separately (Sorsa et al., in preparation). In the present paper we discuss the findings of maternal–child correlations of SCEs in association with the smoking status of the mother.

Results and discussion

Chromosomal damage in passively exposed restaurant personnel

Non-smoking personnel working in indoor restaurants without restrictions of smoking of the public or personnel probably represents one of the groups most heavily exposed to ETS at work. The environmental monitoring data, including analysis of polyaromatic compounds, total particulate matter and genotoxic activity of particulate samples, from typical night restaurant show high levels of contamination of the indoor air (Husgafvel-Pursiainen et al., 1986). The biochemical intake markers of tobacco smoke, i.e., cotinine and thiocyanate, also show significantly increased values in the passively exposed restaurant personnel as compared with non-exposed persons (Husgafvel-Pursiainen et al., 1987). Still, these intake markers are only a few percent of the values found in the actively smoking group of waiters (Fig. 1).

Both chromosome aberrations and SCEs were analysed in a total of 32 subjects (Table 1, Fig. 2). No significant differences were seen between the

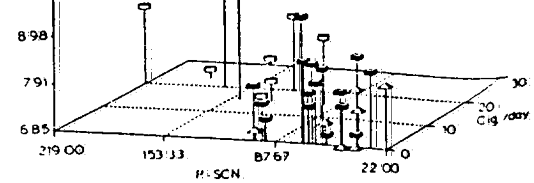

Fig. 1. Dose dependence of tobacco smoke exposure and SCE frequency among restaurant personnel, as evaluated on the basis of individual values in mean SCE/cell, thiocyanates (μmole/l) in plasma (P-SCN) and number of cigarettes smoked daily.

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TABLE 1

MEAN LYMPHOCYTE SCEs AND MEAN PERCENTAGE OF LYMPHOCYTES WITH CHROMOSOME ABERRATIONS IN DIFFERENT SMOKING CATEGORIES

Smoking status	Number of subjects	Mean SCEs /cell (± SD)	% aberrant cells (incl. gaps; ± SD)
Active smokers	9	8.21 ± 1.19	5.0 ± 1.7
Non-smokers			
Passively exposed ex-smokers	9	8.14 ± 0.76	4.6 ± 3.1
Passively exposed never-smokers	7	8.08 ± 0.62	2.7 ± 1.8
Non-exposed	7	7.47 ± 0.59	3.7 ± 1.6

groups or subgroups in the 2 parameters, neither was there any correlation at the individual level. In the total material, SCEs of the smoking waiters were significantly increased in comparison to non-smoking persons in the study (Husgafvel-Pursiainen, 1987). However, a clear trend in the prevalence of chromosome aberrations, especially in chromosome-type aberrations, is seen in the group of ex-smokers (mean non-smoking time after cessation of smoking was 9.6 ± 6.8 years; range 1–23 years) and smokers (Table 2). The difference is significant ($P < 0.05$) when all never-smokers (n = 17) are compared with current and ex-smokers (n = 18). However, in the larger population (Husgafvel-Pursiainen et al., 1987), neither cotinine nor SCE values of passively exposed ex-smokers (U-cot mean 52 ± 27 ng/ml, SCE mean 7.2 ± 1.3, n = 12) differed significantly from the values of never-smokers (U-cot mean 60 ± 44 ng/ml, SCE mean 8.6 ± 1.1, n = 15).

The significance of chromosome-type aberrations and rearrangements has been stressed earlier, since they are independent of age and sex and are

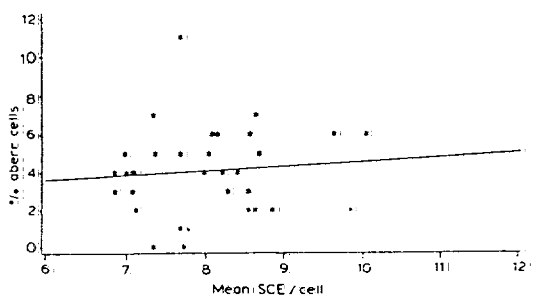

Fig. 2. Relationship of structural chromosome aberrations and SCEs ($r = 0.089$, non-significant) among the group of restaurant personnel (see Fig. 1).

positively correlated with the duration of smoking (Vijayalaxmi and Evans, 1982; Obe et al., 1984; Littlefield and Joiner, 1986). The result also indicates the persistence of lesions leading to chromosome-type aberrations in persons who stopped smoking years ago. In comparison, the SCEs in peripheral lymphocytes of smokers decrease to normal in a few months after stopping smoking (Sarto et al., 1987).

Fetal exposure to tobacco smoke

The hazards of maternal smoking to the normal development of the fetus have long been known (see e.g., National Research Council, 1986). Risks of spontaneous abortion, preterm births, prenatal deaths and low full-term birth weights have been associated with maternal smoking during pregnancy (Surgeon General, 1986). A recent case-control study suggested an association between maternal smoking and increased risk of childhood malignancies (Stjernfeldt et al., 1986). Lowering of birthweight has also been associated with heavy passive exposure of the mother to ETS (Martin and Bracken, 1986; Rubin et al., 1987), and fetal exposure caused by maternal passive smoking has

TABLE 2

NUMBER OF CHROMOSOME-TYPE ABERRATIONS IN SMOKERS, EX-SMOKERS AND NEVER-SMOKERS [a]

	Smokers	Ex-smokers	Never-smokers
Dicentrics	1/900	3/900	1/1400
Other rearrangements	3/900	2/900	1/1400
Breaks	6/900	9/900	7/1400
Total	10/900	14/900	9/1400

[a] Number of aberrant cells/cells analyzed.

been documented by the detection of cotinine in amniotic fluid samples (Andersen et al., 1982).

In the present study, we report the sister-chromatid exchange results in cord-blood samples taken during delivery of actively and passively exposed smokers and non-exposed non-smokers.

Biochemical intake markers measured from maternal peripheral blood just before delivery and from cord blood just after birth of the child showed transplacental concentrations of these tobacco smoke constituents to be at nearly the same level as in the mother. The correlation coefficient of maternal–cord-blood plasma cotinine was $r = 0.81$ ($P < 0.001$; 11 pairs) and for plasma thiocyanate $r = 0.92$ ($P < 0.001$; 17 pairs).

As expected on the basis of earlier studies, smoking mothers had significantly ($P < 0.01$) increased SCE values (range 7.5–10.8; mean 9.0 ± 0.9; $n = 17$) as compared with non-smoking mothers (range 6.7–8.9; mean 8.1 ± 0.9; $n = 25$). A dose–effect correlation was seen both for plasma thiocyanate value and for the number of cigarettes smoked daily (Fig. 3A).

Only 7 of the non-smoking mothers said in the personal interview that they had been exposed to ETS because of spouse's smoking. However, no significant increase of SCEs was detected in this group of passive smokers; the mean was 8.4 ± 1.0 as compared with 7.9 ± 0.9 of the non-exposed non-smoking mothers ($n = 18$).

On the basis of the biochemical intake markers, however, the small group of passively exposed persons were exposed at their homes only minimally. All of the mothers had been out of occupational ETS exposure about one month before delivery during their maternity leave.

The SCE rate in the cord-blood samples was significantly lower (mean $= 6.0 \pm 0.5$; $n = 43$) than in maternal samples (mean $= 8.4 \pm 0.9$; $n = 42$). We reported earlier also (Husgafvel-Pursiainen et al., 1980) that young children (mean age 1.5 years) have significantly lower SCE rates than adults. In earlier studies of smoking mothers and their newborn children no effect of smoking on SCEs was observed in maternal blood, while these studies also reported lower mean SCE rates in cord-blood samples (Ardito et al., 1980; Seshadri et al., 1982). A possible source of discrepancy in the maternal SCE values of smokers vs. non-smokers is the

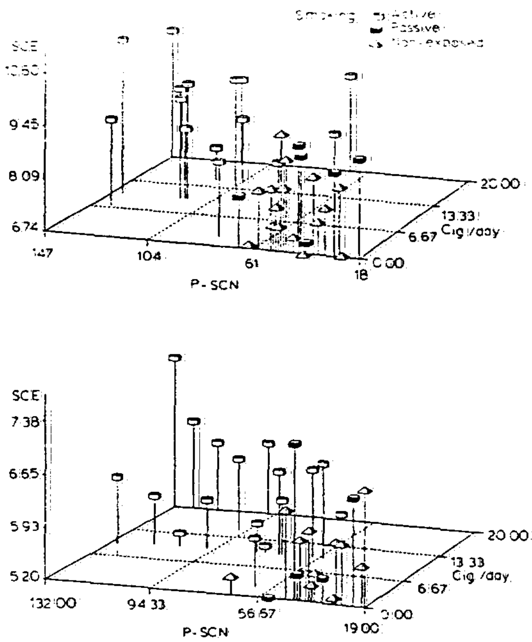

Fig. 3. Association of individual mean SCE to thiocyanate concentration (μmole/l) in plasma (P-SCN) and maternal smoking (number of cigarettes/day) in groups of smoking and non-smoking mothers (A) and newborn children (B); in A the P-SCN and SCE were determined from peripheral blood and in B from cord blood.

bromodeoxyuridine concentration used during culturing, as shown by Lundgren et al. (1987).

In transplacentally exposed children no significant effect of maternal smoking on SCEs was observed (mean 6.1 ± 0.5 in babies of smoking mothers vs. 5.9 ± 0.5 in babies of non-smoking mothers). In the individual values, however, a clear trend of heavy maternal smoking can be seen; children with the highest plasma thiocyanate values tend to have higher SCE rates than children of mild smokers and non-smokers (Fig. 3B), although this difference is not statistically significant. Neither was there any correlation between maternal SCE value and cord-blood value, even though cotinine and thiocyanate concentrations in plasma showed highly significant correlations between maternal and cord-blood samples.

Several hypotheses can be presented to explain the low SCE rate in cord-blood lymphocytes: either the lymphocyte population responding to the

mitogen stimulus is different in its SCE response in children and adults, or transplacental exposure to exogenous SCE-inducing agents differs from the maternal situation with regard to effective concentrations and long enough exposure time during the fetal period.

Recently accumulated evidence associates involuntary exposure to environmental tobacco smoke with health effects in non-smokers; this has been most clearly shown for respiratory organ infections among children exposed to parental smoking and for a small increase of lung cancer risk among non-smoker spouses exposed to ETS caused by the other spouse's smoking (Surgeon General, 1986; National Research Council, 1986). The biochemical intake markers usually show passive smokers' exposure to be only a few percent of the values found among active smokers (see e.g., Jarvis et al., 1984, 1985; Sorsa et al., 1985). In newborn children exposed transplacentally, the biochemical intake markers show values at nearly the same level as in their mothers; still, the fetal exposure time is only a few months.

The cytogenetic damage, well documented among active smokers (see e.g., IARC, 1986), cannot be shown to be associated with involuntary exposure to tobacco smoke in either of the two exposure situations studied, occupational or transplacental. The obvious insensitivity of the cytogenetic parameters may partly be due to their unspecificity in relation to any single compound. Also, tobacco smoke exposure may be confounded by other potentially genotoxic agents in the environment.

Acknowledgements

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SECTION 5

ETS AND LUNG CANCER

ANIMAL STUDIES

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SCOTH REVIEW - VOLUME 3

SECTION 5

ANIMAL STUDIES

Despite the differences between ETS and SS, we are aware of six reports of animal studies which have used aged and diluted SS (ADSS) as an ETS surrogate with short term (14 days) and long term (90 days) exposure in rats and hamsters and which have reported full histopathological and cytogenetic results.

The ADSS concentrations used in these studies were up to twenty times the average indoor ETS concentrations.

The histopathalogical changes observed in rats were limited to epithelial hyperplasia and squamous metaplasia in the rostral nose and the larynx, i.e. typical adaptive responses. All changes were reversible.

Minimal histopathological changes were observed in the trachea or lungs of rats. The changes were reversible post-inhalation, and considered to be typical of an adaptive response to repeat exposure. No histopathological changes were observed in the entire respiratory tract of hamsters. No cytogenetic changes whatsoever were found in the chronic animal studies.

SECTION 5

ANIMAL STUDIES

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Poster

Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study

By L. von Meyerinck[1], G. Scherer[1], F. Adlkofer[1], R. Wenzel-Hartung[2], H. Brune[2] and C. Thomas[3]

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Key words: sidestream smoke, cigarette; inhalation; sidestream smoke; nasal epithelium

Summary

A 90-day feasibility study was performed in which rats and hamsters were exposed to the sidestream smoke of cigarettes. The only histopathological changes observed were hyperplasia and metaplasia of the epithelium covering the dorsal nasal turbinate bones in rats. These effects were reversible within 90 days.

Materials and Methods

Animals species: Male and female Fischer F 344/CrlBr rats and male and female Syrian golden hamsters (body weight at the beginning of the exposure: male rats 240 g, female rats 161 g, male hamsters 108 g, female hamsters 117 g). Animals were kept in steel cages during the exposure and in macrolon cages throughout the remaining time. Animals had no access to food during the exposure.

Animal exposure: Chamber volume 35 m^3. Exposure concentration: 4 mg/m^3 total particulate matter; 25–30 ppm carbon monoxide. Ventilation rate: 6 air changes/h. Exposure time: 10 h/d. 5 d/wk for 90 days; sham-exposed animals served as controls.

Grouping of animals: Exposure groups: 65 male and 35 female rats/65 male and 35 female hamsters; reversibility group: 40 male rats and 40 male hamsters were kept for a further 90 days after termination of exposure. Sham control: 80 male rats, 35 female rats, 80 male hamsters, 35 female hamsters. Room control: 55 male rats, 45 female rats, 55 male hamsters, 50 female hamsters.

Generation of sidestream smoke: Kentucky Reference Cigarettes 2R1F (8 cigarettes simultaneously, 11 puffs/cig.) smoked on a Borgwaldt smoking machine. In order to ensure that the animals were uniformly exposed in terms of age and quantity of smoke, the cages were rotated inside the chamber from day to day.

Chamber monitoring and dosimetry: All determinations for individual parameters were performed according to (1).

Histopathology: The following organs were investigated by light microscopy: nasal cavity, larynx, trachea, lungs, liver, kidneys, adrenal glands, brain, pituitary gland, bladder, heart, thyroid gland, parathyroid gland, thymus, mammary gland, testes and ovaries.

Table 1. Average concentrations of relevant substances in the exposure and sham-exposure chamber.

		Exposure	Sham
Particles	(mg/m^3)	4.3	< 0.1
CO	(ppm)	25	11
NO	(ppm)	400	60
NO_2	(ppb)	70	nd*
Nicotine	($\mu g/m^3$)	1,000	2
Formaldehyde	($\mu g/m^3$)	600	6
Acetaldehyde	($\mu g/m^3$)	1,200	4
Acrolein	($\mu g/m^3$)	450	11
Ammonia	(mg/m^3)	3.1	0.1
Dimethyl-nitrosamine	(ng/m^3)	200	25
Benzo(a)pyrene	(ng/m^3)	56	nd*

nd* = not detectable

Table 2. Dosimetric measurements in smoke-exposed and sham exposed male rats and hamsters at the end of the 90-day exposure period (data are mean ± standard deviation of 16 animals).

	Smoke-exposed		Sham-exposed	
	Rats	Hamsters	Rats	Hamsters
COHb (%)	3.3 ± 0.6	3.1 ± 0.4	0.8 ± 0.5	0.3 ± 0.1
Serum nicotine (ng/ml)	99 ± 40	51 ± 20	3 ± 2	3 ± 3
Serum cotinine (ng/ml)	350 ± 68	115 ± 40	3 ± 3	0.5 ± 1

Results and Conclusions

Table 1 gives the concentrations of relevant substances in both the exposure and the sham chamber. The levels in the exposure chamber were at least 1 and in some instances 2 orders of magnitude higher than reported for smoke polluted rooms under real-life conditions. Table 2 summarizes the results of the dosimetric parameters. COHb-levels and nicotine and cotinine concentrations in serum were similar to or even higher than those obtained in smokers. In rats nicotine levels were twice and cotinine levels were three times higher than in hamsters.

The body weight gain of rats during the exposure and sham exposure phases was similar and did not differ from that of the cage controls. Weight gain was lower in hamsters of both sexes in the exposure and sham exposure groups (probably due to lack of food or to the handling process). After exposure was discontinued, the body weights of the exposed and sham exposed hamster groups attained the same levels as found among the cage control groups (data not shown).

Histophatological changes were detectable in rats only: hyperplasia and squamous metaplasia in the nasal cavity were found in both female and male rats after 90 days of exposure to tobacco smoke (fig. 1a). Thirty days after the exposure had ceased, these changes partially receded (fig. 1b) and they were completely reversed within 60 days after exposure (fig. 1c). All other parts of the respiratory tract (larynx, trachea, bifurcation and lung) showed no alterations.

Whereas rats treated with diesel exhaust exhibit a persistent overload of the lungs with diesel

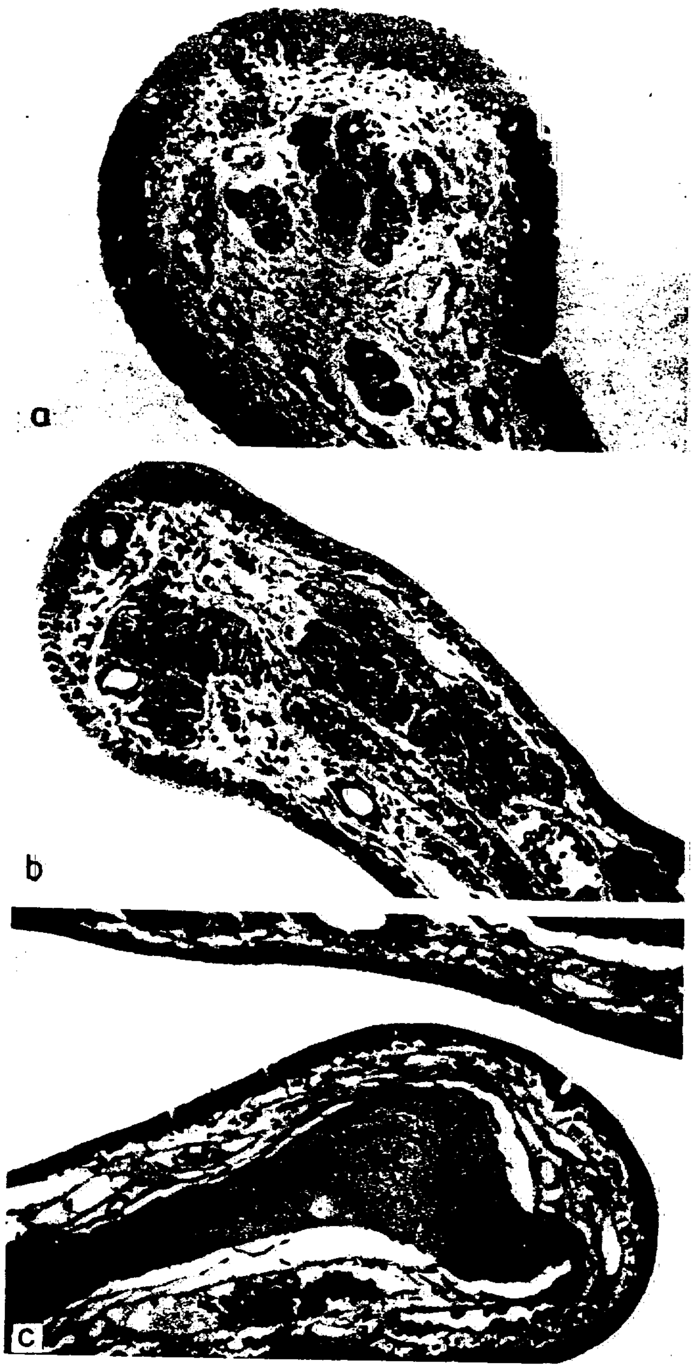

Fig. 1. Histopathological changes in the nasal cavity of male Fisher F 344 rats. For an explanation see text.

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soot even after short-time exposure (2), particles from tobacco smoke seem to be effectively removed from the bronchial tract by the pulmonary clearing mechanism. The continuous overload with diesel soot might cause chronic damage of the bronchial epitelium and an accelerated cell turnover rate thus enhancing the DNA adduct formation (3) and probably also DNA misrepair. In view of this it is doubtful whether a long-term animal experiment with tobacco smoke may lead to an increased cancer rate in rats. A long-term animal experiment with tobacco smoke under the conditions of the diesel exhaust studies is feasible.

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Further references can be obtained from the authors.

Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette

I. Inhalation Toxicology and Histopathology

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Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette[1]

I. Inhalation Toxicology and Histopathology

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Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette. I. Inhalation Toxicology and Histopathology. COGGINS, C. R. E., AYRES, P. H., MOSBERG, A. T., OGDEN, M. W., SAGARTZ, J. W., AND HAYES, A. W. (1992). *Fundam. Appl. Toxicol.* 133–140.

Sprague–Dawley rats were exposed 6 hr per day for 14 consecutive days to aged and diluted sidestream smoke (ADSS), used as a surrogate for Environmental Tobacco Smoke (ETS), at concentrations of 0.1 (typical), 1 (extreme), or 10 (exaggerated) mg of particulates per cubic meter. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End-points included histopathology, CO-oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The only pathological response observed was slight to mild epithelial hyperplasia and inflammation in the most rostral part of the nasal cavity, in the high-exposure group only. No effects were noted at medium or low exposures. The minimal changes noted were reversible, using a subgroup of animals kept without further treatment for an additional 14 days. Overall, the end-points used in the study demonstrated that there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations and that the activity was only minimal at very exaggerated concentrations (particle concentrations 100 times higher than typical real-world concentrations). © 1992 Society of Toxicology.

Numerous statements have been made in the scientific literature on the biological activity of environmental tobacco smoke (ETS: Department of Health and Human Services, 1986; National Research Council, 1986). This study was designed to obtain information on the effects seen in animals exposed to aged and diluted sidestream smoke (ADSS), at concentrations equivalent and higher than those reported for ETS in the field. Measurements of the latter vary significantly and usually include contributions of suspended particulates other than ETS (DHHS, 1986). Consequently, the ADSS target concentrations were chosen as 0.1, 1, and 10 mg/m^3. Respectively, these correspond to typical, extreme (or "worst-case"), and exaggerated field measurements (Oldaker *et al.*, 1990).

The primary end-point of this study was the histopathology of the respiratory tract and related organs, as assessed by a pathologist with experience in rodent inhalation studies with cigarette smoke. The aims were to determine whether biological responses could be observed after exposure to ADSS at different concentrations, what these observed responses were, and the no-observed-effect-level (NOEL) for the study. An additional objective was to study the reversibility of any changes observed after exposure to ADSS. To study this recovery process, subgroups of animals were kept for an additional 2 weeks without treatment, at the end of the 14-day exposure.

Other end-points included DNA adducts and alveolar macrophage cytogenetics; these are reported separately (Lee *et al.*, 1992).

MATERIALS AND METHODS

Experimental design. The experimental design was based on published guidelines (OECD, 1981). Three groups of animals were exposed to ADSS; there was a sham-exposed group exposed only to filtered air. Animals were exposed to smoke inside whole-body chambers, using nose-only restraint tubes. A further group of animals was kept as chamber controls (without tube restraint) and as sentinels for the detection of disease. There were 48 animals per sex in each of the five groups. Animals were exposed 6 hr per day for 14 consecutive days. The groups were "stagger-started" at intervals of 1 day. Animals in satellite groups were kept for a further 14 days without treatment to assess reversibility.

Experimental animals. A total of 560 (280 male and 280 female) animals, weighing 125–150 g was purchased from Charles River Laboratories (Raleigh, NC). Animals were housed individually in transparent polycarbonate cages and acclimated to laboratory conditions for 14 days prior to the first exposure.

The Sprague–Dawley rat (Crl:CD/BR, VAF/Plus) was chosen as the experimental animal because it has frequently been used in inhalation studies

[1] Presented at the 1992 Annual Meeting of the Society of Toxicology, Seattle, WA.

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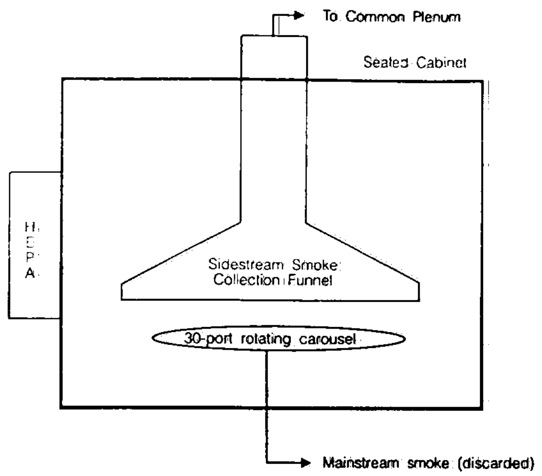

FIG. 1. Apparatus used to collect sidestream smoke from reference cigarettes.

and there is a large amount of background inhalation data available in the scientific literature.

Within 5 days of delivery, 5 animals per sex were randomly chosen and killed for collection of sera, which was tested for the following antibodies to disease: Reovirus Type 3, cilia associated respiratory bacillus, Kilham's rat virus, Toolan's H-1 virus, pneumonia virus of mice, Sendai, rat coronavirus/sialodacryoadenitis virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. Antibody testing was made on sera obtained from 5 animals per sex at the beginning and end of the inhalation part of the experiment, and at reversibility (total of 20 animals per sex). The lungs from the sentinel animals were taken and examined histopathologically to ascertain health status.

Within a week of delivery, the animals were allocated into five groups each of 48 animals per sex, such that the body weights in the groups were as homogeneous as possible. The mean (±SD; $n = 240$) weight of males at randomization was 142.1 ± 7.9 g; in females the mean was 148.4 ± 7.9 g.

During the week after allocation into groups, animals were tail-tattooed (Animal Identification and Marking Systems, Piscataway, NJ) with their permanent identification number.

The animals were housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Subpart E, *Specifications for the Humane Handling, Care, Treatment, and Transportation of Warm-Blooded Animals Other Than Dogs, Cats, Rabbits, Hamsters, Guinea Pigs and Non-human Primates*. Reference was also made to the DHHS document *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23).

Animals had unrestricted access to certified feed (Purina Rodent Chow No. 5002: presented as pellets) and distilled water. No feed was available during inhalation exposures. Feed was withheld overnight prior to necropsy. Chemical analyses of feed, water, or bedding were not performed, because it was deemed unlikely that contaminants would adversely affect the experiment.

Cigarettes. The 1R4F reference cigarettes were purchased from the Tobacco and Health Research Institute (Lexington, KY). A full description of the mainstream, sidestream, and ETS chemistry of the 1R4F cigarette has been given elsewhere (R. J. Reynolds, 1988).

Aerosol generation apparatus. A 30-port smoke generator (CH Technologies, Westwood, NJ) similar to that described by Baumgartner and Coggins (1980) and by Ayres *et al.* (1990) was fitted with an aluminum cone (Fig. 1) for collection of sidestream smoke (SS). The smoke generator was placed in a sealed cabinet with a HEPA filter attached. Mainstream smoke was generated under Federal Trade Commission conditions (except butt length: seven puffs were taken instead of burning to a fixed butt length) and was discarded. Sidestream smoke was drawn from the cone into a common plenum, using 3-in.-diam (76.2 mm) PVC tubing throughout. Different amounts of SS were drawn from the plenum for each chamber and mixed with dilution air drawn from the room through HEPA filters (Fig. 2). Room air was HEPA-filtered "upstream." The sham and sentinel animals were kept in chambers that were not attached to the plenum.

Animal exposure apparatus. The whole-body inhalation chamber has been described previously (Moss *et al.*, 1982) and is available commercially (Lab Products, Maywood, NJ). Each chamber was operated at a flow rate of 16 cubic feet/min (in excess of 15 air changes/hr).

The conical nose-only restraint tubes (Baumgartner and Coggins, 1980) are also commercially available (CH Technologies, Westwood, NJ); they were used to minimize contamination of the pelt with deposited ADSS which could then be absorbed dermally or ingested during preening (Langård and Nordhagen, 1980). Tubes for males were 73 mm in diameter and 263 mm long; for females the diameter was 62 mm and the length 168 mm. Both tube types had inlets 22 mm in diameter. The ventilation slots on the restraint tubes were covered with duct tape.

On exposure days, individual animals were taken from their cage in the chamber, placed inside a nose-only restraint tube, and replaced in the tube into the same cage. The orientation of the tube was such that urine and feces passed from the tube, through the wire floor of the cage, and onto paper-lined catch pans placed under each of the trays.

Daily characterization of inhalation exposures. During animal exposures, probes were used to monitor the aerosol presented. This monitoring was by collection of aerosol on glass-fiber pads followed by gravimetric determination of collected particulates using Cahn C-31 microbalances (Cahn, Cerritos, CA). The RAM-1 instrument was used to give an on-line estimate of particulate concentrations.

The main analytical instrument used for carbon monoxide (CO) and carbon dioxide (CO_2) was the Horiba PIR-2000 (Horiba Instruments, Inc., Irvine, CA), calibrated daily with certified gas mixtures (AIRCO Welding Supply, Greensboro, NC). Oxygen concentrations (%) were monitored by a Horiba PMA-200 instrument, also calibrated with a certified gas mixture. Very low concentrations of CO were measured with the Miran 80 gas analyzer (Foxboro Instruments, S. Norwalk, CT). Data from the on-line instruments were logged manually every 60 min.

Measurement of nicotine and 3-ethenylpyridine was by gas chromatography with thermionic-specific detection (Ogden, 1991). Chamber atmo-

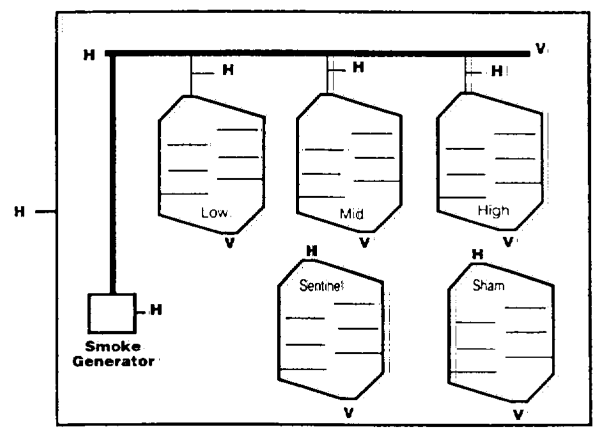

FIG. 2. Layout of laboratory used to expose animals to aged and diluted sidestream smoke. V, vacuum; H, HEPA filter.

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spheres were sampled with XAD-4 sorbent tubes (SKC, Inc., Eighty-Four, PA) which were extracted for analysis with ethyl acetate containing 0.01% triethylamine (Ogden, 1989).

Solanesol measurements were made by liquid chromatography with UV detection at 205 nm (Ogden and Maiolo, 1990). Following gravimetric determination of particulates, the glass-fiber pads were extracted for analysis with 3 ml methanol.

Measurement of particle size distribution was made three times during the study, using a Mercer-style cascade impactor (Mercer *et al.*, 1970; In-Tox Products, Albuquerque, NM). The impactor had cut-off diameters in the range of 0.4–2.5 μm under the conditions of use (1.2 liters/min); calculation of mass median aerodynamic diameter was by probit analysis. The coverslips (uncoated) used to collect the aerosol for impactor analysis were weighed using Cahn C-31 microbalances.

Air temperature and RH of the aerosol were measured with a condensation dew point hygrometer (Model 1100 DP, General Eastern Instrument Co., Watertown, MA).

Clinical observations. Animals were inspected visually for signs of overt toxicity as they were being transferred from their cages to the restraint tubes, and when being transferred back to their cages. More detailed clinical observations were made on each animal once every 4 days, before the exposure and within 2 hr of the end of the exposure.

Body weights. Individual body weights were determined within 48 hr of receipt, at randomization, and every 4 days thereafter, using Mettler PM 2000 balances.

Dosimetry. Blood samples were obtained after 6 hr of smoke exposure, on different exposure days throughout the experiment. Blood was drawn from the retro-orbital sinus, using anesthesia with 70% CO_2 in air and heparinized micropipettes, and held on ice in plastic cuvettes containing disodium edetate during the time between sampling and analysis.

Blood COHb concentrations were determined on 0.5 ml of the total sample, using a Model 482 CO-Oximeter (Instrumentation Laboratories, Hartford, CT). Subsamples of the blood collected for COHb were taken for the determination of plasma nicotine and cotinine. The latter analyses were performed by an ELISA method.

Necropsy. Animals selected at random were killed on the day following their last exposure, and the time interval was recorded. Feed was not available to the animals during this time interval.

At necropsy, animals were weighed and then killed by first anesthetizing with 70% CO_2 in air and then exsanguination via the vena cava prior to cessation of heartbeat. Blood samples for the various assays to be performed were collected from the vena cava.

Animals were subjected to a complete gross examination in the presence of a board-certified veterinary pathologist, with special attention paid to the respiratory tract.

Clinical pathology. The following assays were performed on whole blood obtained at each necropsy: red blood cell count, hemoglobin, hematocrit, mean red cell volume, mean red cell hemoglobin, mean red cell hemoglobin concentration, white cell count, differential white cell counts, reticulocyte counts, and platelet count. The anticoagulant Na_2-EDTA was used; standard hematological methods were used.

The following assays were performed on serum obtained from animals at each necropsy: calcium, phosphorus, chloride, sodium, potassium, glucose, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, urea nitrogen, albumin, creatinine, total bilirubin, total cholesterol, triglycerides, and total protein. The time of blood sampling was recorded. Sure-Sep II serum separators (Organon-Teknika, Durham, NC) were used to minimize hemolysis; the time between blood collection and serum collection was kept as short as possible. Standard analytical methods were used.

Organ weights. The lungs (complete with trachea but excluding the larynx), brain, liver, testes (pair), kidneys (pair), and heart (excluding major vessels) were weighed at each necropsy using Mettler PM 460 balances. Organ weights and the (fasted) body weight recorded immediately before death were used to calculate organ:body weight ratios. The time from removal of the organ until weighing was minimized and tissues were kept in saline until they were weighed.

Tissue collection. Tissues were removed from each animal and fixed in 10% neutral buffered formalin (NBF), at a volume dilution of 1 part tissue to at least 15 parts formalin (Feldman and Seely, 1988). The fixative contained 20 ml of 1% eosin per 20 liters of 37% formalin, as a precaution to identify the fluid as fixative. Lungs were infused with NBF at a volume that ensured proper fixation. The trachea was ligated after inflation.

The following tissues were collected: adrenals, aorta, bone (sternum; femur), brain, cecum, colon, cranium, duodenum, epididymides, esophagus, eyes/optic nerve, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes, mammary glands, nasopharynx, nose/turbinates, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle (thigh), skin (abdominal), spinal cord (lumbar), spleen, stomach, tail, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, Zymbal's gland.

Histopathology. Respiratory tract tissues (nasal passages, larynx, trachea, conducting airways, deep lung), heart and related lymph nodes (thymic and peribronchial), and gross changes were examined in each of 28 animals per sex randomly allocated to histopathology in each of the four exposure groups (20 for the 14-day necropsy and 8 for the reversibility necropsy).

The nasal tissues were cut at three different locations to obtain representative sections of the different epithelia, as described previously (Young, 1981). The lungs were sectioned to provide a section along the main stem bronchus of each lung lobe. A precise anatomical site for cutting the larynges is required: serial-step sections were taken to reach this site (Burger *et al.*, 1989; Sagartz *et al.*, 1992).

Tissues were stained with hematoxylin and eosin (H&E); duplicate slides of a representative section of the anterior nasal tissues, larynx, lung, and trachea were stained with periodic acid–Schiff/Alcian blue to facilitate evaluations of mucus-secreting cells. Tissues were read by an ACVP board-certified veterinary pathologist, with knowledge of the treatment groups.

Statistical analyses. Statistical evaluations were made using Bartlett's test of homogeneity of variance, followed by analysis of variance techniques. The statistical evaluation of incidence and severity data for histopathology was made by the Kolmogorov–Smirnov test (Siegel, 1956). Statistical tests were carried out to 5%, two-sided criteria.

RESULTS

Inhalation Exposures

The mean concentrations ($\pm$SD; $n = 14$) of wet total particulate matter (WTPM) for the low-, medium-, and high-exposure groups were 0.09 ± 0.01, 1.08 ± 0.08, and 9.79 ± 0.59 mg/m^3, respectively. Figure 3 shows the within- and between-day variation in WTPM concentrations. These WTPM exposures resulted in CO concentrations (ppm) of 3.61 ± 0.81, 11.3 ± 1.6, and 57.0 ± 3.8.

Nicotine concentrations ($\pm$SD, $n = 14$) at the medium and high exposures were 252 ± 57 and 1708 ± 346 $\mu g/m^3$. Although nicotine could be detected in the low-exposure chambers, the values were unexpectedly low: the mean was 0.91 ± 0.28 $\mu g/m^3$. The ratios of nicotine to WTPM were 1.01 ± 0.27, 23.4 ± 5.1, and 17.4 ± 3.4% for low, medium, and high exposures, respectively. The values for 3-ethenylpyridine at the low, medium, and high exposures were 1.30 ± 0.34, 36.0 ± 3.0, and 242 ± 26 $\mu g/m^3$; these data resulted in ratios of 3-ethenylpyridine to nicotine of 147 ± 36, 14.8 ± 3.3, and 14.7 ± 3.4% for low, medium, and high exposures, respectively.

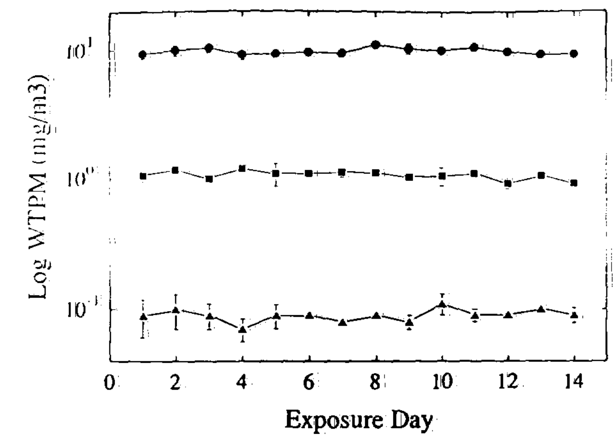

FIG. 3. Variation in daily concentrations of wet total particulate matter in aged and diluted sidestream smoke presented to experimental animals. Means ± standard deviations. Targets were 0.1, 1, and 10 mg/m³.

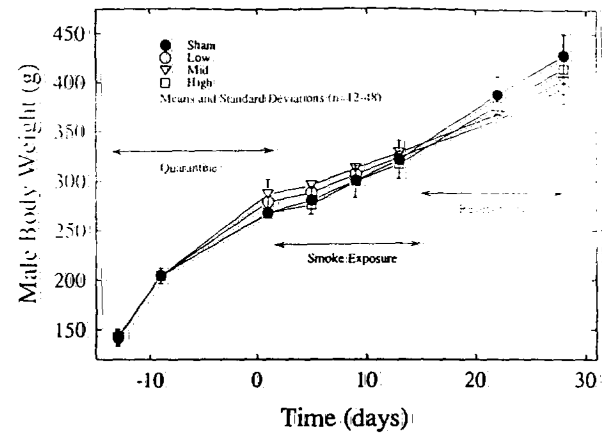

FIG. 4. Change in body weight of male animals.

Solanesol concentrations (±SD, $n = 14$) in the low, medium, and high exposures were 1.93 ± 0.24, 25.1 ± 2.3, and 186 ± 18 $\mu g/m^3$, respectively; the ratios of solanesol to WTPM were 2.16 ± 0.32, 2.33 ± 0.11, and $1.90 \pm 0.11\%$.

None of the above analytes could be detected at significant concentrations in the sham chamber or in the exposure room. In the exposure room, the mean CO_2 concentration (±SD, $n = 16$) was 403 ± 20 ppm; in the sham chamber the mean (±SD, $n = 14$) concentration was 1307 ± 58 ppm. The mean concentrations (±SD, $n = 14$) in the low, medium, and high chambers were 1522 ± 101, 1458 ± 166, and 1842 ± 100 ppm, respectively.

Particle size distributions at the medium and high exposures were similar, with the average values (three observations) being 0.42 and 0.29 μm, respectively. The standard geometric deviations were 1.52 and 1.80. Insufficient material was obtained during the 6 hr of the low exposure for determination of particle size distribution.

In-Life Observations

There were no treatment-related clinical signs or mortalities and the serology results were negative. Animals in the smoke-exposed groups showed body weights that were similar to those in the sham group (Figs. 4, 5).

Blood COHb concentrations at the end of the exposures were negligible in the sham low-exposure groups. The means for the medium and high groups (sexes combined, ±SD, $n = 30$) were 0.91 ± 0.46 and $5.80 \pm 0.94\%$, respectively.

The ELISA assay had limits of detection (LOD) of 4.5 ± 3.3 ng/ml for nicotine and 6.9 ± 7.6 ng/ml for cotinine. The sham and low-exposure groups had plasma nicotine concentrations at or below the LOD; the medium-exposure group had plasma nicotine concentrations above the LOD but below the limit of quantification. The mean plasma nicotine for the high exposure (sexes combined, ±SD, $n = 30$) was 43.6 ± 13.5 ng/ml.

The sham and low-exposure groups had plasma cotinine concentrations at or below the LOD. The means for the medium and high exposure (sexes combined, ± SD, $n = 29$) were 43.2 ± 15.7 and 323 ± 161 ng/ml, respectively.

Necropsy Data

There was no effect of exposure on terminal body weight: the mean values (± SD, $n = 21$–24) in males for sham, low, medium, and high exposures at the 14-day necropsy were 287 ± 18, 299 ± 25, 300 ± 21, and 288 ± 17 g, respectively. In females, the means were 212 ± 12, 211 ± 15, 210 ± 15, and 204 ± 11 g. At the recovery necropsy, the means (±SD, $n = 12$–16) for males were 380 ± 50, 363 ± 34, 368 ± 39, and 377 ± 46 g for the sham, low, medium, and high exposures, respectively; for the females, the values were 251 ± 15, 259 ± 14, 250 ± 20, and 247 ± 24 g.

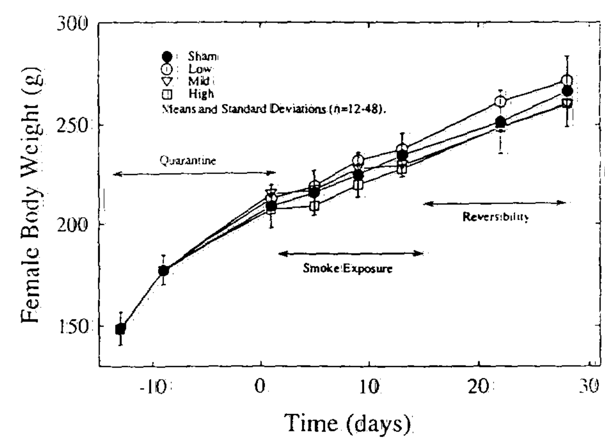

FIG. 5. Change in body weight of female animals.

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FIG. 6. Low-power view of a transverse section of the rat nasal passages, immediately caudal to the incisor teeth. The arrow marks the position of the change shown in Figs. 7 and 8. H&E; original magnification, ×45.

There were no significant differences between the groups for any clinical pathology parameters at either of the necropsies.

There were no treatment-related gross observations at either necropsy; there were no differences in organ weights.

Histopathology

In the Nasal I section (transverse, immediately caudal to the incisor teeth), there was *chronic active inflammation*, in the high-exposure groups only. This change mainly involved the dorsal nasal conchae (nasoturbinates) and the adjacent wall of the middle meatus (Fig. 6). In this lesion, the inflammatory infiltrate consisted of lymphohistiocytic cells admixed with small numbers of polymorphonuclear leukocytes. Figure 7 shows a high-power view of the tip of a nasal concha from a typical sham animal; Fig. 8 shows a similar region from a typical high-exposure-group animal. Figure 9 shows the distributions of the different severities of this change for the four groups. The distribution of the change in the high-exposure group was significantly different from that in the other groups; there were no such significant differences at the low or medium exposures.

The above lesion was typically found in association with, and in the same anatomic site as, *epithelial hyperplasia*. In this latter change, there was hypercellularity and thickening of the respiratory epithelium of the dorsal nasal conchae and the adjacent wall of the middle meatus. Figure 10 shows the

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FIG. 7. High-power view of the tip of a nasal concha (nasoturbinate) from a typical sham-exposed rat. The epithelium is two to three cell layers thick. H&E; original magnification, ×500.

distribution of the different severities of this change for the ... groups. The distribution of the change in the high-exposure group was significantly different from that in the other groups; no such significant effect was noted at the low or medium exposures.

There were no other histopathological changes noted in animals killed at the 14-day necropsy, nor were any changes noted in the animals killed at the reversibility necropsy (i.e., the minimal changes noted at the end of the exposure were totally reversible).

DISCUSSION

The results obtained here are in agreement with earlier work (von Meyerinck *et al.*, 1989), where only a single concentration of ADSS was used (4 mg/m^3) for 10 hr/day. In this latter work, the main histopathological change noted was also in the rostral nasal cavity, a change which appears morphologically to be very similar to that noted in the high-exposure group in the present study, although the overall duration was much less (14 days instead of 90). It is thus possible that the change does not progress in subchronic exposures.

The analytical results described here show clearly that solanesol is a good marker for the particulate phase of ADSS, there being a constant ratio between solanesol measured by liquid chromatography and gravimetric estimates of WTPM

FIG. 8. High-power view of the tip of a nasal concha (nasoturbinate) from a typical high-exposure rat. The epithelium is five to six cell layers thick. Note the presence of an inflammatory infiltrate, consisting of lymphohistiocytic cells admixed with small numbers of polymorphonuclear leukocytes. H&E; original magnification, ×500.

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(mean ratio 2.1%). This ratio is in very good agreement with ratios for true ETS generated by human smokers in a controlled environment. Ratios of solanesol to particulates from the 1R4F cigarette in these environments range from 2.6 to 4.0% with a mean ratio of 3.2% (Ogden, unpublished data). The slightly lower ratios noted in this study can be attributed to the smoke generation and particulate analysis systems used. Sidestream smoke generated only by a smoking machine results in slightly lower solanesol concentrations than does human smoking which includes sidestream smoke and exhaled mainstream smoke (Ogden, unpublished data). In addition, particulates were determined as WTPM in this study which, by definition, includes water. Significant moisture condensation on the particles will deflate the solanesol/WTPM ratio and apparently does so at the high exposure used here. Measurement of ETS particulates is not appreciably affected by trapped water due to the high dilution of the smoke aerosol. Likewise, water does not appear to contribute significantly to the WTPM concentrations measured at the low and medium exposures.

The surprisingly low nicotine concentrations in the low-exposure chamber have not yet been explained. One hypothesis is that nicotine is selectively adsorbed on the large surface area of the chamber or the connecting plumbing. This reasoning is consistent with known adsorption characteristics of nicotine in a stainless-steel environmental chamber (Thome *et al.*, 1986). Due to the unusual decay characteristics of nicotine in some situations, 3-ethenylpyridine (a product of nicotine combustion) is gaining acceptance as a tracer for ETS vapor phase (Ogden, 1991).

The exposure levels used in this study were carefully chosen with respect to typical concentrations in real-life environments. The low exposure used (target and actual particulate concentrations of 0.10 and 0.09 mg/m^3, respectively)

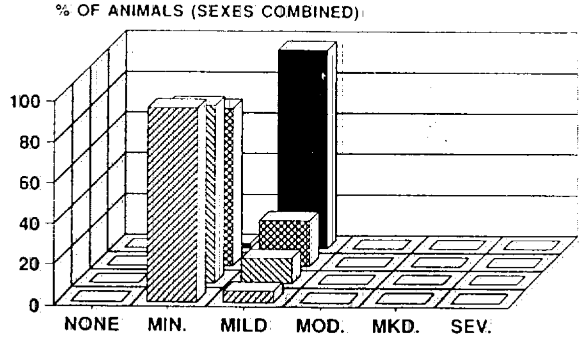

FIG. 9. Distribution of severities of chronic active subepithelial inflammation in Nasal I in the different groups at the 14-day necropsy (sexes combined). MIN., minimal change; MILD, mild change; MOD., moderate change; MKD., marked change; SEV., severe change. (□) Sham, (□) low, (▨) medium, (■) high.

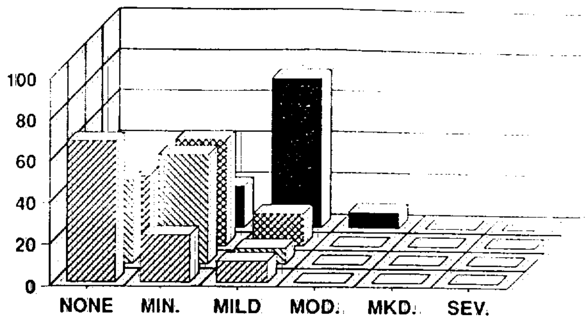

FIG. 10. Distribution of epithelial hyperplasia in Nasal I in the different groups at the 14-day necropsy (sexes combined). MIN., minimal change; MILD, mild change; MOD., moderate change; MKD., marked change; SEV., severe change. (□) Sham, (□) low, (▨) medium, (■) high.

is typical of exposure concentrations in public places where smoking is allowed without restriction. For example, average concentrations of tobacco smoke indicators in restaurants are 10.5 $\mu g/m^3$ nicotine; 2.5 $\mu g/m^3$ 3-ethenylpyridine (Ogden, unpublished data); and 0.108 mg/m^3 particulates and 1.5 $\mu g/m^3$ solanesol (Oldaker *et al.*, 1990). These concentrations are equivalent to the concentrations generated in the low exposure (excepting the anomaly noted for the nicotine concentration). As an example of more extreme conditions, the highest concentration of solanesol ever measured in a real-life situation is 12.8 $\mu g/m^3$ (with an associated particulate concentration of 0.355 mg/m^3), recorded in a smoke-filled billiard parlor (Ogden and Maiolo, 1989). This concentration is approximately half that measured in the medium exposure of this study.

The results presented here show only a minimal effect of exposure to very high concentrations of aged and diluted sidestream smoke; the only effect being the completely reversible (in 14 days) changes seen in the rostral nasal cavity at the high exposure only. Since the concentrations of smoke used were gross exaggerations (100-fold) of any reasonable field situation for ETS (DHHS, 1986; Oldaker *et al.*, 1990), we conclude that ETS, at typical and maximal concentrations, is unlikely to have any significant toxicological activity in tests similar to those used here. The NOEL for the study is 1 mg/m^3.

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Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette

II. DNA Adducts and Alveolar Macrophage Cytogenetics

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The chemical constituents of cigarette smoke are greatly diluted in environmental tobacco smoke (ETS). In the typical indoor environment where cigarettes are smoked, the mean value of respirable suspended particles is approximately 0.1 mg/m^3. In this study, we used aged and diluted sidestream smoke (ADSS) of 1R4F University of Kentucky research cigarettes as a surrogate for ETS and exposed Sprague–Dawley rats nose-only to 0, 0.1, 1.0, and 10 mg wet total particulate matter (WTPM)/m^3 for 6 hr per day for 14 consecutive days. DNA from lung, heart, larynx, and liver was tested for adduct formation after 7 and 14 days of exposure and after 14 days of recovery. In addition, alveolar macrophages from animals exposed for 7 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any of the animals at 0.1 or 1.0 mg WTPM/m^3, which represent ambient and 10-fold exaggerated ETS concentrations, respectively. Slight diagonal radioactive zones, characteristic of adducts observed in human smokers and in animals exposed to mainstream smoke, were observed, but only in lung and heart DNA of animals exposed to the highest concentration of ADSS (10 mg WTPM/m^3), a 100-fold exaggeration of typical field measurements of ETS. The mean relative adduct labeling values (±SE) were 8.7 (±0.2) adducts per 10^9 nucleotides for lung DNA and 5.7 (±0.7) adducts per 10^9 nucleotides for heart DNA after 14 days of exposure. No elevation in chromosomal aberrations was observed in alveolar macrophages. These results indicate a no-observed-effect-level (NOEL) of 1.0 mg/m^3 for DNA adduct formation in lung and heart and a NOEL of at least 10 mg/m^3 for the induction of chromosome aberrations in alveolar macrophages under the conditions of this study. © 1992 Society of Toxicology.

The biological significance of exposure to environmental tobacco smoke (ETS) has emerged as a subject of intense public discussion. ETS consists of sidestream smoke (SS), emitted from the burning end of a cigarette between puffs, and exhaled mainstream smoke (MS). Condensate from both MS (Doolittle *et al.*, 1990a; Lee *et al.*, 1990b) and SS (Doolittle *et al.*, 1990b) have been reported to be genotoxic *in vitro*. Several *in vitro* studies (Claxton *et al.*, 1989; Lewtas *et al.*, 1987; Ling *et al.*, 1987; Lofroth *et al.*, 1988; Lofroth and Lazardis, 1986) have reported that concentrated ETS particulate matter is mutagenic. Although cigarette smoke or its condensate is positive in many genotoxicity endpoints, its potency is very low compared to other complex mixtures to which humans are exposed, such as coke oven emissions, roofing tar, and diesel exhaust (Williams and Lewtas, 1985). Recently, Lewtas *et al.* (1991) estimated that the tumor-initiating potencies for the extractable organic matter from coke oven emissions and urban air are 1000- and 100-fold higher, respectively, than that of cigarette smoke condensate (CSC). They also concluded that the levels of DNA adduct formation with these complex mixtures correlated with tumor potency. In marked difference to concentrated smoke, constituents in ETS are highly diluted. Therefore, the critical question is not whether highly concentrated ETS is genotoxic *in vitro* but whether ETS, at its normal concentration range, is genotoxic *in vivo*.

The measurement of DNA adducts by the ^{32}P-postlabeling assay (Randerath *et al.*, 1981; Reddy and Randerath, 1986) is one of the best ways to assess DNA damage following exposure to complex mixtures. It is generally accepted that the majority of genotoxic chemicals are converted metabolically to electrophiles which attack nucleophilic centers in nucleic acids and proteins, resulting in the formation of covalent adducts (Miller and Miller, 1981; Hemminki, 1983). Therefore, DNA adducts serve as an indicator of exposure to genotoxic chemicals. The P_1 nuclease enhancement version of the ^{32}P-postlabeling assay (Reddy and Randerath, 1986) is sufficiently sensitive to detect one adduct per 10^{-9} to 10^{-10} nucleotides and therefore is ideally suited for complex mixtures of unknown chemical composition. Using this

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assay, several studies have reported DNA adducts in rodents following inhalation exposure to cigarette smoke (Gupta *et al.*, 1989; Bond *et al.*, 1989; Gairola and Gupta, 1991) as well as after skin painting with CSC (Randerath *et al.*, 1988; Reddy and Randerath, 1990). Adduct assays have been extended to include other complex mixtures such as diesel exhaust (Wong *et al.*, 1986; Bond *et al.*, 1989, 1990; Gallagher *et al.*, 1990), coal soot (Gallagher *et al.*, 1990), and lubricating oil (Schoket *et al.*, 1989). However, studies on the formation of DNA adducts in experimental animals after cigarette smoke exposure at or near the concentrations found in ETS have not been reported.

Recently, Rithidech *et al.* (1989) reported that pulmonary alveolar macrophages (PAM) of rats exposed to mainstream cigarette smoke developed chromosomal aberrations, thus demonstrating the usefulness of these cells as a tool for studying the cytogenetic effects of cigarette smoke in the rat lung.

In the present study, aged and diluted sidestream smoke (ADSS) was used as a surrogate for ETS. Cytogenetic damage in PAM and covalent DNA adducts in several internal organs of rats were assessed following exposure to different concentrations of ADSS for 14 consecutive days, 6 hr/day. The results demonstrate that chromosomes in PAM are not damaged and that adducts are formed only in the lungs and heart of animals exposed to the highest concentration of ADSS (10 mg WTPM/m^3). DNA adducts were not detectable at 0.1 mg WTPM/m^3, a typical concentration of human ETS exposure, or at a 10-fold higher concentration of 1.0 mg WTPM/m^3.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from New England Nuclear (Wilmington, DE). Micrococcal nuclease (100–200 units/mg) and nuclease P_1 (255 units/mg) were obtained from Sigma (St. Louis, MO). Calf spleen phosphodiesterase (2 units/mg) was obtained from Boehringer–Mannheim (Indianapolis, IN). Polyethyleneimine (PEI)-cellulose-coated thin-layer chromatographic (TLC) sheets (Machery Nagel) were obtained from Brinkmann Scientific (Westbury, NY). T_4 polynucleotide kinase was obtained from GIBCO-BRL (Gaithersburg, MD).

Experimental animals. Experimental animals have been described elsewhere (Coggins *et al.*, 1992). Briefly, 5-week-old Sprague–Dawley rats (Charles River, Raleigh, NC) were acclimated for 2 weeks prior to exposure in 2-cubic-meter stainless-steel inhalation chambers. The animal rooms had controlled lighting (12 hr dark and 12 hr light), temperature (20–24°C), and humidity (40–60% relative humidity). Animals were allowed unrestricted access to feed (Purina Rodent Chow 5002) and distilled water, except during the smoke exposures.

Smoke generation and exposure conditions. Smoke generation and exposure conditions have been described (Coggins *et al.*, 1992). Briefly, smoke was generated from 1R4F research cigarettes with a 30-port AMESA generator (CH Technologies, Westwood, NJ) fitted with an aluminum cone for collection of sidestream smoke. Cigarettes were smoked according to the Federal Trade Commission method (a 35-ml puff of 2 sec duration, once/min) except that instead of a fixed butt length, a fixed number of puffs (seven) was taken (Baumgartner and Coggins, 1980; Ayres *et al.*, 1990). Mainstream smoke was exhausted and discarded and the sidestream smoke was drawn into a common plenum. Different amounts of sidestream smoke were provided for each chamber and mixed with dilution air drawn from the animal room through HEPA filters. Target concentrations for suspended particulates were 0, 0.1, 1, and 10 mg/m^3. Exposures were for 6 hr per day, for 14 consecutive days.

Experimental design. For the DNA adduct assay, rats were divided into four groups: sham, low (0.1 mg WTPM/m^3), medium (1.0 mg WTPM/m^3), and high exposures (10.0 mg WTPM/m^3). Each group contained 12 male animals. Four animals in each group were killed after 7 and 14 days of exposure for DNA adduct analysis. The remaining 4 animals in each group were kept for a further 14 days without smoke exposure for the reversibility study.

DNA isolation and digestion. Whole lung, heart, liver, and larynx tissues were weighed and homogenized in Hanks' balanced salt solution (HBSS; Ca^{2+}- and Mg^{2+}-free). Homogenates were centrifuged and the supernatant extracts discarded. Pellets were resuspended in HBSS. DNA was isolated on a Genepure 341 nucleic acid purification system (Applied Biosystems, Inc., Foster City, CA) by solvent extraction and enzymatic digestion of protein and RNA. DNA concentration was estimated spectrophotometrically (1 A_{260} = 50 μg DNA/ml). Absorbance ratios (260/280) of all DNA samples ranged from 1.6 to 1.8. Samples of DNA were digested essentially as described by Gupta *et al.* (1982) with minor modifications. Five to 10 μg of DNA was digested for 3.5 hr at 37°C in a total volume of 10 μl containing 0.6 U of micrococcal nuclease and 5.0 μg of spleen phosphodiesterase (dialyzed 24 hr against water) in 100 mM $CaCl_2$ and 200 mM sodium succinate buffer, pH 6.0.

^{32}P-postlabeling assay. The nuclease P_1 procedure (Reddy and Randerath, 1986) was used as described with slight modifications. DNA digest samples were incubated for 45 min with nuclease P_1 under the following conditions: to 10 μl of DNA digest was added 4 μl of a solution containing each of the following: 2.0 μl of nuclease P_1 (4 μg/μl), 0.7 μl of 1 mM $ZnCl_2$, 0.7 μl of 0.4 M sodium acetate, pH 5.0, and 0.6 μl of distilled, deionized water. Immediately following nuclease P_1 treatment, 2.0 μl of 10 mM Ches–NaOH, pH 9.6, was added. DNA digests then were reacted with 75 μCi of [γ-^{32}P]ATP using 4.5 U of T_4 polynucleotide kinase and kinase buffer (200 mM Ches–NaOH, 100 mM $MgCl_2$, 100 mM dithiothreitol, and 10 mM spermidine, pH 9.6). Separation of the ^{32}P-labeled adducts was on PEI-cellulose TLC sheets using the following solvents: D1, 1.0 M sodium phosphate, pH 6.0 (overnight onto a wick); D3, 5.3 M lithium formate, 8.5 M urea, pH 3.5; D4, 1.2 M lithium chloride, 8.5 M urea, 0.5 M Tris base, pH 8.0 (onto a wick); D5, 1.7 M sodium phosphate, pH 6.0 (overnight onto a wick).

Quantification of DNA adducts. For each batch of [^{32}P]ATP, the specific activity was determined by measuring the kinase-catalyzed incorporated of radioactivity into 10 pmol of 2'-deoxyadenosine 3'-monophosphate (Reddy and Randerath, 1986). Values of specific activity fell within the range of 1.5 to 2.5×10^6 CPM/pmol. TLC maps were wrapped in Mylar plastic and scanned for 8 hr using the AMBIS radioanalytic imaging system (AMBIS Systems, Inc., San Diego, CA) under an argon atmosphere. A 3.2×3.2-mm resolution plate was used. The counting efficiency averaged approximately 20%. The AMBIS radioanalytic imaging system is a computer-controlled imaging system which directly quantifies radiation. Its use in ^{32}P-postlabeling assay of DNA adducts has been reported (Turteltaub *et al.*, 1990). The system has a two-dimensional proportional detector composed of 952 elements that simultaneously detect multiple β emissions. Sample patterns are replicated in the resulting computer picture which is displayed as a high-resolution image on a video monitor. Results are stored in permanent data files. Computer-generated images of the adduct maps were marked and scored for radioactivity. Background from the adjacent area was subtracted. The amount of adducts present in each sample was calculated from the radioactivity present in adduct spots or zones and the specific activity of the [γ-^{32}P]ATP used, and expressed as total number of adducts/10^9 nucleotides. In our experience, the images produced on the monitor equal, and often exceed, those of conventional autoradiography. The relative adduct labeling (RAL) values calculated from AMBIS-generated data were compared with those obtained by autoradiography and liquid scintillation counting. Results were comparable.

DISCUSSION

The P_1 nuclease enhancement version of the ^{32}P-postlabeling assay (Reddy and Randerath, 1986) is the most sensitive method available for evaluating DNA adducts following *in vivo* exposure to complex mixtures. DNA adducts have been observed in experimental animals and humans following exposure to a variety of complex mixtures including mainstream cigarette smoke. For example, the presence of DNA adducts in smokers has been reported, with significantly lower levels in ex-smokers and nonsmokers (Phillips *et al.*, 1988, 1990; Randerath *et al.*, 1989; Cuzick *et al.*, 1990). In rodents, adduct formation has been reported in lungs and other respiratory tissues (Bond *et al.*, 1989; Gupta *et al.*, 1989; Gairola and Gupta, 1991) following smoke exposure. On the basis of these reports, the rat appears to be a suitable animal model for molecular dosimetry of covalent DNA binding at low concentrations of complex mixtures such as ETS.

DNA adducts generally decrease and eventually disappear upon cessation of exposure to genotoxic agents. Adducts in ex-smokers are reported to be significantly less than in current smokers and eventually reach the levels of nonsmokers (Phillips *et al.*, 1988; Randerath *et al.*, 1989). In baboons exposed to cigarette smoke, DNA adducts disappeared with time during smoking cessation (Marshall *et al.*, 1991). However, the rate of decrease may depend on the tissues involved. Slower disappearance of polycyclic aromatic hydrocarbon–DNA adducts in lung compared to skin has been reported (Schoket *et al.*, 1989). In the present study, 2 weeks were insufficient to show a complete disappearance of the adducts in either lung or heart from the high-exposure animals. This finding is consistent with observations reported by others. A decrease in adduct levels was not observed until 4 weeks after the cessation of diesel exhaust exposure to rats (Bond *et al.*, 1990). Lung DNA adducts from cigarette-smoke-exposed rats remained at the same level 1 week after cessation of exposure with a significant decrease after 19 weeks (Gupta *et al.*, 1989).

Rithidech *et al.* (1989) exposed animals to approximately 100 to 200 mg TPM/m^3 for 6 hr/day, 5 days/week for 22 to 24 days and reported an increase in the frequencies of chromosomal aberration in exposed animals. Other cytogenetic studies on smoke-exposed rats (Lee *et al.*, 1990a; Basler, 1982) and hamsters (Korte *et al.*, 1981), however, failed to induce chromosome aberration, sister chromatid exchanges, or micronuclei formation in bone marrow and lymphocytes. Based on these results, PAM appears to be a more sensitive tissue for measuring the clastogenic potential of inhaled tobacco smoke than is bone marrow. In our study, none of the exposed animals, including the highest exposure group, yielded PAM with increased chromosome aberration.

Concentrations of ETS in indoor environments vary, but average concentrations of respirable suspended particles are approximately 0.1 mg/m^3 (Oldaker, 1989; Oldaker *et al.*, 1990a,b). A weakly visible DRZ in lung and heart tissues was observed in rats after exposure to ADSS, but only at the highest exposure (10 mg WTPM/m^3). Although smoke-related DNA adducts have been reported in the larynx in human smokers (Randerath *et al.*, 1989) and in rats exposed to mainstream smoke (Gairola and Gupta, 1991), our study did not detect DNA adducts in this organ or liver at the high exposure (10 mg WTPM/m^3), a 100-fold exaggerated ETS concentration. It is significant that concentrations (0.1 and 1.0 mg WTPM/m^3), which represent an average level of ETS in most places where smoking is allowed and a 10-fold increase in concentration, respectively, did not result in any exposure-related adducts. Even in tissues with visible DRZ, the level of adducts was near the detection limit. None of the concentrations tested significantly increased chromosome aberrations in PAM. Thus, under the conditions of these studies, 1.0 mg WTPM/m^3 represents a no-observed-effect-level (NOEL) for DNA adduct formation in lung and heart and a NOEL of at least 10 mg/m^3 exists for the induction of chromosome aberrations in alveolar macrophages.

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In vitro modification of DNA with CSC. One milligram of lung DNA isolated from Sprague–Dawley rats was treated with 5 mg (in 500 μl of DMSO) of CSC from Kentucky Reference 1R4F cigarettes in the presence of an S9 metabolic activation system. The S9 liver homogenate (obtained from Molecular Toxicology, Annapolis, MD) was prepared according to Ames *et al.* (1975). The S9 concentration in the mix was 5% (v/v) and 5.0 ml of the S9 mix was added to the reaction tube. The reaction mixture was incubated for 5 hr at 37°C. DNA was extracted with the ABI Genepure extractor.

In vivo modification of DNA with benzo[a]pyrene. Female B6C3F1 mice were given a single ip dose of 40 or 60 mg/kg of benzo[*a*]pyrene B[*a*]P in 0.1 ml of DMSO/corn oil (50/50 v/v). Livers were collected 43 hr after B[*a*]P administration. DNA was extracted by the A.S.A.P. genomic DNA isolation kit (Boehringer–Mannheim).

Pulmonary alveolar macrophage isolation and analysis of chromosomal aberration. Immediatley following the seventh consecutive daily ADSS exposure period, animals were injected ip with colchicine (6 mg/kg, 12 mg/ml colchicine stock solution). Four hours later, the animals were killed by asphyxiation with 70% carbon dioxide (CO_2) and the tracheas were cannulated with 14G iv catheters. The lungs and heart were removed as a block and placed in ice cold HBSS without Mg^{2+} or Ca^{2+} for 30 to 60 min. The lungs were lavaged repeatedly through the cannula using a 10-ml syringe filled with 7 ml of ice-cold HBSS. After the lungs were filled, they were massaged for 1 min and the lavage fluid was removed and placed in a 50-ml centrifuge tube on ice. Lavaging and massaging continued until approximately 50 ml of lavage fluid was collected. The lavage fluid was centrifuged and the supernatant fraction drawn off. The pellet was resuspended in prewarmed (37°C) 75 mM KCl and incubated at 37°C for 25 min. After centrifugation, the cells were fixed first with 6:1 absolute MeOH:glacial acetic acid and then three more times with Carnoy's fixative (3:1 absolute MeOH: glacial acetic acid). Metaphase spreads were prepared. Two slides per animal were stained for 7 min with Giemsa diluted 1:20 (10 ml Giemsa, 190 ml distilled H_2O) and mounted with coverslips using Depex mounting medium.

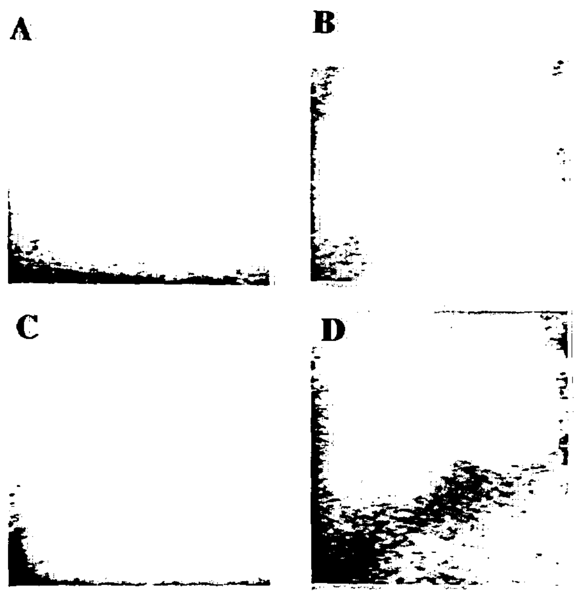

FIG. 1. Comparison of TLC maps of lung DNA from Sprague–Dawley rats exposed to aged and diluted sidestream smoke for 14 days: (A) sham exposure; (B) low exposure (0.1 mg WTPM/m³); (C) medium exposure (1.0 mg WTPM/m³); (D) high exposure (10 mg WTPM/m³). TLCs were scanned for 8 hr with AMBIS radioanalytic imaging system.

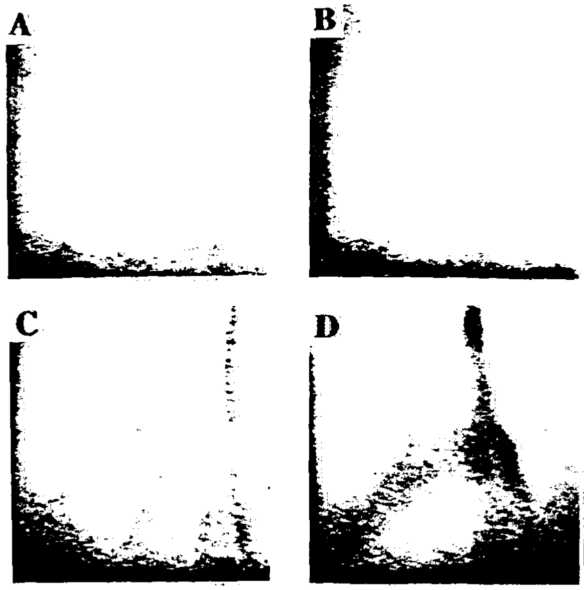

FIG. 2. Comparison of TLC maps of heart DNA from Sprague–Dawley rats exposed to aged and diluted sidestream smoke for 14 days: (A) sham exposure; (B) low exposure (0.1 mg WTPM/m³); (C) medium exposure (1.0 mg WTPM/m³); (D) high exposure (10 mg WTPM/m³). TLCs were scanned for 8 hr with AMBIS radioanalytic imaging system.

Fifty metaphases for each animal were scored for chromosome aberrations where possible. Sometimes 50 scorable cells were not available. Scarcity of scorable cells is not a sign of toxicity, but is due to the very low mitotic index of PAM (Pinkett *et al.*, 1966; Evans and Bills, 1969). Scoring was done without knowledge of treatment group.

Statistical analysis. Statistical significance ($p < 0.05$) was evaluated by analysis of variance for DNA adduct data and by Fisher's exact test for chromosomal aberration data.

RESULTS

After 14 exposure days, no overt signs of toxicity or significant body weight differences were observed between any of the exposed groups and the sham controls. Exposed lungs were not pigmented and the only histopathological changes observed were mild hyperplasia and inflammation of the nasal cavity in the high-exposure group only (Coggins *et al.*, 1992). These changes were reversible.

^{32}P maps of DNA samples from lung and heart tissues from animals in the highest exposure group (10 mg WTPM/m³) exhibited slight diffuse diagonal radioactive zones (DRZ) extending from close to the origin into the center of the chromatogram. Lung DNA from animals in the highest exposure group (10 mg WTPM/m³) exhibited DRZ after 7 and 14 days of exposure and the DRZ was still present after a 14-day recovery period. Heart DNA also exhibited faint but recognizable DRZs in the highest exposure group but only after 14 days of exposure and after the 14-day recovery period. Adduct maps of lung and heart DNA from the four

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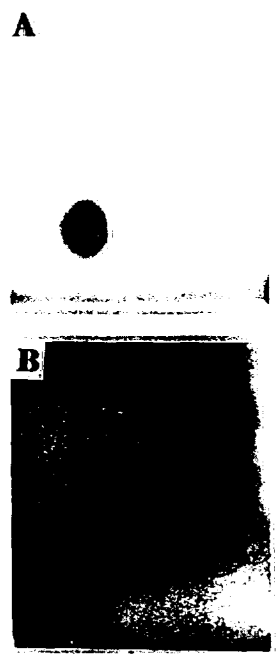

FIG. 3. TLC maps of B[a]P-DNA adduct from liver of mouse injected ip with 40 mg/kg of B[a]P (A) and CSC-DNA adduct prepared *in vitro* (B). TLCs were scanned for 8 hr with AMBIS radioanalytic imaging system. The RAL values are 839 adducts per 10^9 nucleotides for B[a]P-DNA adducts and 55 adducts per 10^9 nucleotides for CSC-DNA adducts.

exposure groups are shown in Figs. 1 and 2, respectively. The liver and larynx did not exhibit exposure-related adducts at any concentration or time point. Maps of CSC-DNA adducts from *in vitro* reactions and B[a]P-DNA adducts from liver of mice injected ip with B[a]P are shown in Fig. 3. Mean RAL values of the four organs (lung, heart, liver, and larynx) after 14 days of exposure are presented in Fig. 4.

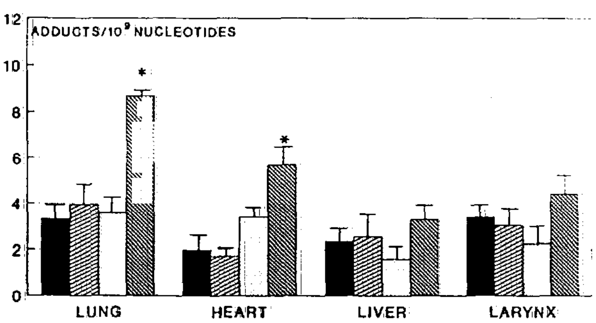

FIG. 4. RAL values (mean ± SE; $n = 4$) of DNA adducts in tissues of Sprague-Dawley rats after a 14-day exposure to a high concentration (10 mg WTPM/m^3) of aged and diluted sidestream smoke. Differences between the groups were tested by ANOVA. *Significantly different ($p < 0.05$) from sham control. (■) Sham; (▨) low, 0.1 mg WTPM/m^3; (□) medium, 1 mg WTPM/m^3; (□) high, 10 mg WTPM/m^3.

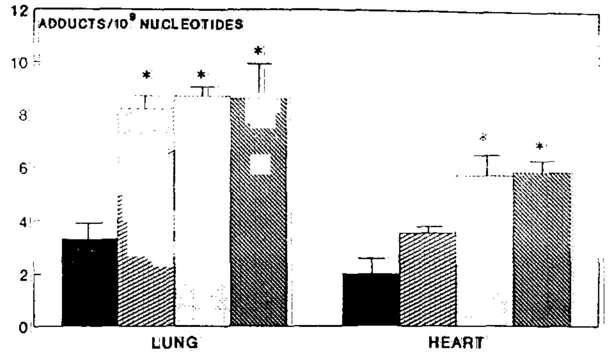

FIG. 5. Comparison of RAL values (mean ± SE, $n = 4$) of DNA adducts in lung and heart after 7- and 14-day exposures to high concentration (10 mg WTPM/m^3) of aged and diluted sidestream smoke and after a 14-day recovery period. Differences between the groups were tested by ANOVA. *Significantly different ($p < 0.05$) from sham control. (■) Sham, (▨) 7 days, (□) 14 days, (□) 14-day recovery.

Only the lungs and heart from the highest smoke exposure (10 mg/m^3) contained adduct concentrations significantly higher than sham controls. The RAL values were 7 to 10 adducts per 10^9 nucleotides (21 to 30 amol/μg DNA) for lung and 4 to 7 adducts per 10^9 nucleotides (12 to 21 amol/μg DNA) for heart. In both lung and heart, the amount of adducts was similar at the end of the 14-day exposure period and after the 14-day reversibility period (Fig. 5).

The results of the chromosomal aberration assay in PAM after 7 consecutive exposure days are presented in Table 1. Positive control animals injected with cyclophosphamide exhibited a statistically significant increase in chromosomally aberrant cells; however, no statistically significant increase in cells with aberrations was observed in smoke-exposed animals.

TABLE 1
Chromosome Aberration Analysis in Pulmonary Alveolar Macrophages of Rats Exposed to Aged and Diluted Sidestream Smoke for 7 Days

Treatment	Number of animals	Number of cells analyzed	Number of aberrant cells	Percentage cells with aberrations
Sham exposure	3	150	2	1.3
Low exposure (0.1 mg WTPM/m^3)	4	189	2	1.1
Medium exposure (1.0 mg WTPM/m^3)	3	77	0	0
High exposure (10 mg WTPM/m^3)	3	150	6	4.0
Cyclophosphamide (10 mg/kg)	2	89	12	13.5*
Saline (0.5 ml/kg)	3	150	1	0.7

* Statistically significant ($P < 0.05$).

SUBCHRONIC INHALATION STUDY IN RATS USING AGED AND DILUTED SIDESTREAM SMOKE FROM A REFERENCE CIGARETTE

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Male Sprague-Dawley rats were exposed 6 hr/day, 5 days/week for up to 13 weeks to aged and diluted sidestream smoke (ADSS), used as a surrogate for environmental tobacco smoke (ETS), at concentrations of 0.1 ("typical"), 1 ("extreme"), or 10 ("exaggerated") mg of particulates/m^3. Subgroups of animals were killed after 1 and 4 weeks of exposure. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End points included histopathology, CO oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The target particulate concentrations were achieved; at the exaggerated exposure they resulted in CO concentrations in excess of 50 ppm. Particle size distributions showed that the aerosols were completely respirable: the mass median diameter values were less than 1 μm. The only pathological response observed was slight to mild epithelial hyperplasia in the rostral nasal cavity, in the exaggerated exposure group only. No effects were noted at low (typical of measured real-world ETS concentrations) or extreme exposures. The changes were similar in animals killed after 4, 28, or 90 days, and were also similar to those noted in an earlier experiment with only 14 days duration, indicating that the change does not progress with increased exposure duration from 4 to 90 days. The nasal change was absent in a subgroup of animals kept without further smoke exposure for an additional 90 days, indicating complete reversibility. Overall, the end points used in the study demonstrated that (1) there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations, and (2) the activity was only minimal at exaggerated concentrations in one region of one organ only. Based on the nasal histopathology, the NOEL for the 90-day study is > 1 mg/m^3.

The authors thank Jerry Avalos, Jim Corn, Delma France, and Keith Shreve for constructing the inhalation laboratory and for performing the inhalation exposures; Jessica Baker for the animal care; Dr. Michael Ogden and Katherine Maiolo for the nicotine, solanesol, and 3-ethenylpyridine measurements; Sheri Reynolds for serology and the COHb assay; Leroy Gerald for necropsy; Dr. Kuo-Mei Chang for the plasma nicotine analyses; and Mark Forsell for the histology.

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INTRODUCTION

Numerous statements have been made in the scientific literature on the biological activity of environmental tobacco smoke (ETS) (Department of Health and Human Services, 1986; National Research Council, 1986). Recent work with animals exposed 6 hr/day for 14 consecutive days to aged and diluted sidestream smoke (ADSS) reported minimal histopathological changes in such studies, even when smoke concentrations many times higher than those reported in the field were used (Coggins et al., 1992). ADSS has been shown to be a suitable surrogate for ETS (Baker and Proctor, 1990; Gori and Mantel, 1991; Guerin et al., 1992).

The purpose of the present study was to determine for histopathology (1) if the changes seen in the above study of 14 days duration were modified in subchronic (90-day) exposures, (2) whether any new changes were observed in subchronic exposures, (3) whether the dose–response relationship was different after the subchronic exposure (using the same target ADSS concentrations as used previously), and (4) whether the 90-day changes (if any were noted) were reversible in a further 90-day period without any exposure.

Target ADSS concentrations were 0.1, 1, and 10 mg/m^3, the same as those used in the 14-day study. Respectively, these correspond to "typical," "extreme," and "exaggerated" field measurements (Guerin et al., 1992).

The primary end point of this study was the histopathology of the respiratory tract and related organs, as assessed by pathologists with experience in rodent inhalation studies with cigarette smoke. Secondary end points included those suggested by regulatory authorities (Organisation for Economic Co-Operation, 1981); other end points (CO-oximetry, plasma nicotine and cotinine) were added to the basic protocol to verify that smoke inhalation did occur (dosimetry).

MATERIALS AND METHODS

Experimental Design

The experimental design was based on published guidelines (Organisation for Economic Co-Operation, 1981). Three groups of animals were exposed to ADSS; there was a sham-exposed group exposed only to filtered air. Animals were exposed to smoke inside whole-body chambers (Chen et al., 1989) using nose-only restraint tubes. A further group of animals was kept as sentinels for the detection of disease. There were 106 male animals in each of the four groups. Animals were exposed 6 hr/day, with animals killed after 4, 28, or 90 days of exposure. The 4-day exposures were performed with only 10 animals per chamber; the chambers were refilled with the remaining 96 animals per group for the 28-

and 90-day exposures. Animals in satellite groups were kept for a further 90 days without treatment to assess reversibility.

Experimental Animals

A total of 506 male animals, weighing 125–150 g, was purchased from Charles River Laboratories (Raleigh, NC). Animals were housed individually in transparent polycarbonate cages and acclimated to laboratory conditions for 14 days prior to the first exposure.

The Sprague-Dawley rat (Crl:CD/BR, VAF/Plus) was chosen as the experimental animal because it has frequently been used in inhalation studies and there is a large amount of background inhalation information available in the scientific literature. Males only were used, to allow sufficiently large group sizes for the many different end points studied. Earlier work (Coggins et al., 1992) showed no difference between the responses shown by males and females.

Within 5 days of delivery, five animals were randomly chosen and killed for collection of sera, which were tested for the following antibodies to disease: reovirus type 3, cilia associated respiratory bacillus, Kilham's rat virus, Toolan's H-1 virus, pneumonia virus of mice, Sendai, rat coronavirus/sialodacryoadenitis virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. Antibody testing was made on sera obtained from five animals at the beginning, midpoint, and end of the inhalation part of the experiment, and at reversibility (total of 25 animals). The lungs from the sentinel animals were taken and examined histopathologically to ascertain health status.

Within a week of delivery, the animals were allocated into four groups of 106 animals each, such that the body weights in the groups were as homogeneous as possible. The mean ($\pm$SD, n = 106) weights in the sham, low, mid, and high groups at randomization were 157.7 $\pm$ 6.6, 157.5 $\pm$ 6.7, 157.6 $\pm$ 6.3, and 157.7 $\pm$ 6.6 g, respectively.

During the week after allocation into groups, animals were tail-tattooed (Animal Identification and Marking Systems, Piscataway, NJ) with their permanent identification number.

The animals were housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Sub-part E, *Specifications for the humane handling, care, treatment, and transportation of warm-blooded animals other than dogs, cats, rabbits, hamsters, guinea pigs and non-human primates*. Reference was also made to the DHHS document *Guide for the Care and Use of Laboratory Animals* (NIH publication 86-23). Animals were housed in a vivarium that had controlled lighting (12 hr of darkness, from 18:00 hr), temperature (20–24°C), and humidity (40–60% RH). Seven-day continuous recordings were kept of RH and temperature. Empty animal rooms

were certified (Certek, Raleigh, NC) as being class 100 (less than 100 particles/m^3.

Animals had unrestricted access to certified feed (Purina Rodent Chow no. 5002, presented as pellets) and distilled water. No feed was available during inhalation exposures. Feed was withheld overnight prior to necropsy. Chemical analyses of feed, water, or bedding were not performed because it was deemed unlikely that contaminants would adversely affect the experiment.

Cigarettes

The 1R4F reference cigarettes were purchased from the Tobacco and Health Research Institute (Lexington, KY). A full description of the mainstream, sidestream, and ETS chemistry of the 1R4F cigarette has been published elsewhere (R. J. Reynolds, 1988), as has a description of the physical and chemical characteristics of mainstream and environmental tobacco smoke (Gori and Mantel, 1991). A description of the origins of ETS has also been published (Baker and Proctor, 1990).

Aerosol Generation Apparatus

Methods have been described in detail elsewhere (Coggins et al., 1992); the following constitutes a basic description only. A 30-port smoke generator (CH Technologies, Westwood, NJ), similar to that described by Baumgartner and Coggins (1980) and by Ayres et al. (1990), was fitted with an aluminum cone for collection of sidestream smoke (SS). Mainstream smoke was generated under Federal Trade Commission conditions (except butt length: 7 puffs were taken instead of burning to a fixed butt length) and was discarded. Sidestream smoke was drawn from the cone into a common plenum used for aging and diluting the smoke, using 3-in. (76.2-mm)-diameter polyvinylchloride (PVC) tubing throughout. Different amounts of SS were drawn from the plenum for each chamber and mixed with further amounts of dilution air drawn from the room through HEPA filters. Room air was HEPA-filtered "upstream." The sham and sentinel animals were kept in chambers that were not attached to the plenum.

Animal Exposure Apparatus

The whole-body inhalation chamber (Moss et al., 1982) is available commercially (Lab Products, Maywood, NJ). Each chamber was operated at a flow rate of 16 ft^3/min (in excess of 15 air changes/hr).

The conical nose-only restraint tubes (Baumgartner and Coggins, 1980) are also commercially available (CH Technologies, Westwood, NJ); they were used to minimize contamination of the pelt with deposited ADSS which could then be absorbed dermally or ingested during preen-

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ing (Langård and Nordhagen, 1980). Tubes were 73 mm in diameter and 263 mm long; the inlet was 22 mm in diameter. The ventilation slots on the restraint tubes were covered with duct tape.

On exposure days, individual animals were taken from their cage in the chamber, placed inside a nose-only restraint tube, and replaced in the tube into the same cage. The positions of racks within the chambers were changed daily in order to minimize any effects of cage position within the chamber.

Daily Characterization of Inhalation Exposures

During animal exposures, probes were used to monitor the aerosol presented. This monitoring was by collection of aerosol on 25-mm Teflon pads (TF-450, Gelman, Ann Arbor, MI), followed by gravimetric determination of collected particulates using Cahn C-31 microbalances (Cahn, Cerritos, CA). The RAM-1 instrument (MIE Corp., Bedford, MA) was used to give an on-line estimate of particulate mass concentrations.

The main analytical instrument used for estimating CO and CO_2 concentrations was the Horiba PIR-2000 (Horiba Instruments, Irvine, CA), calibrated daily with certified gas mixtures (AIRCO Welding Supply, Greensboro, NC). Oxygen concentrations (%) were monitored by a Horiba PMA-200 instrument, also calibrated with a certified gas mixture. Very low concentrations of CO were measured with the Miran 80 gas analyzer (Foxboro Instruments, South Norwalk, CT). Data from the on-line instruments were logged manually every 60 min.

Measurement of nicotine and its pyrolysis product 3-ethenylpyridine (Guerin et al., 1992) was by gas chromatography with thermionic-specific detection. Chamber atmospheres were sampled with XAD-4 sorbent tubes (SKC Inc., Eighty-Four, PA) that were extracted for analysis with ethyl acetate containing 0.01% triethylamine (Ogden, 1989).

Solanesol measurements were made by liquid chromatography with UV detection at 205 nm. After gravimetric determination of particulate mass, the pads were extracted with 3 ml methanol for solanesol analyses (Ogden and Maiolo, 1992).

Measurements of particle size distribution were made with Mercer-style cascade impactors (Mercer et al., 1970; In-Tox Products, Albuquerque, NM). The impactor had cutoff diameters in the range of 0.4–2.5 μm under the conditions of use (1.2 l/min); calculation of mass median diameter and associated standard geometric deviation was by probit analysis. The cover slips (uncoated) used to collect the aerosol for impactor analysis were weighed using Cahn C-31 microbalances.

Temperature and % RH of the exposure atmosphere were measured with a condensation dew point hygrometer (model 1100 DP, General Eastern Instrument Co., Watertown, MA).

Clinical Observations

Animals were inspected visually for signs of overt toxicity as they were being transferred from their cages to the restraint tubes and when being transferred back to their cages. More detailed clinical observations were made on each animal once every 4 days, before the exposure and within 2 hr of the end of the exposure.

Body Weights

Individual body weights were determined within 48 hr of receipt, at randomization, and every week thereafter, using Mettler PM 2000 balances.

Dosimetry

Blood samples were obtained after 6 hr of smoke exposure, on different exposure days throughout the experiment. Blood was drawn from the retro-orbital sinus, using anesthesia with 70% CO_2 in air and heparinized micropipettes, and held on ice in plastic cuvettes containing disodium edetate (Na_2-EDTA) during the time between sampling and analysis.

Blood carboxyhemoglobin (COHb) concentrations were determined on 0.5 ml of the total sample, using a model 482 CO-oximeter (Instrumentation Laboratories, Hartford, CT). Subsamples of the blood collected for COHb were taken for the determination of plasma nicotine and cotinine. The latter analyses were performed by an enzyme-linked immunosorbent assay (ELISA) method (Chang et al., 1992).

Necropsy

The following numbers of animals were allocated to necropsy and subsequent histopathology in each of the four exposure groups: 10 for the 4-day necropsy, 10 for the 28-day necropsy, 25 for the 90-day necropsy, and 15 for the reversibility necropsy. Remaining animals were used for end points other than histopathology (no data presented here).

Animals were killed on the day following their last exposure, and the time interval recorded. Feed was not available to the animals during this interval.

At necropsy, animals were weighed and then killed first by anesthetization with 70% CO_2 in air and then exsanguination via the vena cava prior to cessation of heartbeat. Blood samples for the various assays to be performed were collected from the vena cava.

Animals were subjected to a complete gross examination in the presence of a board-certified veterinary pathologist, with special attention paid to the respiratory tract.

Clinical Pathology

The following assays were performed on whole blood obtained at each necropsy: red blood cell count, hemoglobin, hematocrit, mean red cell volume, mean red cell hemoglobin, mean red cell hemoglobin concentration, white cell count, differential white cell count, reticulocyte count, and platelet count. The anticoagulant Na_2-EDTA was used; standard hematological methods were used.

The following assays were performed on serum obtained from animals at each necropsy: calcium, phosphorus, chloride, sodium, potassium, glucose, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, urea nitrogen, albumin, creatinine, total bilirubin, total cholesterol, triglycerides, and total protein. The time of blood sampling was recorded. Sure-Sep II serum separators (Organon-Teknika, Durham, NC) were used to minimize hemolysis; the time between blood collection and serum collection was kept as short as possible. Standard analytical methods were used.

Organ Weights

The lungs (complete with trachea but excluding the larynx), brain, liver, testes (pair), kidneys (pair), and heart (excluding major vessels) were weighed at each necropsy using Mettler PM 460 balances. Organ weights and the (fasted) body weight recorded immediately before death were used to calculate organ/body weight ratios. The time from removal of the organ until weighing was minimized and tissues were kept in saline until they were weighed.

Tissue Collection

Tissues were removed from each animal and fixed in 10% neutral buffered formalin (NBF), at a volume dilution of 1 part tissue to at least 15 parts formalin (Feldman and Seely, 1988). The fixative contained 20 ml of 1% eosin per 20 l of 37% formalin as a precaution to identify the fluid as fixative. Lungs were infused with NBF at a volume that ensured proper distention and fixation. The trachea was ligated after distention.

The following tissues were collected: adrenals, aorta, bone (sternum, femur), brain, cecum, colon, cranium, duodenum, epididymides, esophagus, eyes/optic nerve, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (various), nasopharynx, nose/turbinates, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle (thigh), skin (abdominal), spinal cord (lumbar), spleen, stomach, tail, testes, thymus, thyroid, tongue, trachea, urinary bladder, and Zymbal's gland.

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Histopathology

Respiratory tract tissues (nasal passages, larynx, trachea, conducting airways, deep lung), heart and related lymph nodes (thymic and peribronchial), and gross changes were examined in each of the animals allocated to histopathology.

The nasal tissues were cut at three different locations to obtain representative sections of the different epithelia, as described previously (Young, 1981). The lungs were sectioned so as to provide a section along the main stem bronchus of each lung lobe. A precise anatomic site for cutting the larynges is required: serial step sections were taken to reach this site (Burger et al., 1989; Sagartz et al., 1992).

Tissues were stained with hematoxylin and eosin (H&E); duplicate slides of a representative section of the anterior nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS-AB) to facilitate evaluations of mucus-secreting cells. Tissues were read by an ACVP board-certified veterinary pathologist with knowledge of the exposure groups.

Statistical Analyses

Statistical evaluations were made using Bartlett's test of homogeneity of variance, followed by analysis-of-variance (ANOVA) techniques. The statistical evaluation of incidence and severity data for histopathology was made by the Kolmogorov–Smirnov test (Siegel, 1956). Statistical tests were carried out to 5%, two-sided criteria.

RESULTS

Inhalation Exposures

The mean concentrations ($\pm$SD, $n = 69$) of wet total particulate matter (WTPM) for the low-, medium-, and high-exposure groups were 0.105 ± 0.013, 1.01 ± 0.071, and 10.3 ± 0.691 mg/m^3, respectively. Figure 1 shows the within- and between-day variation in WTPM concentrations. These WTPM exposures resulted in CO concentrations (ppm) of 2.9 ± 0.59, 9.3 ± 1.87, and 55.1 ± 5.0.

Nicotine concentrations ($\pm$SD, $n = 14$) at the medium and high exposures were 272 ± 65 and 2377 ± 393 μg/m^3. Although nicotine could be detected in the low-exposure chambers, the values were unexpectedly low: the mean was 0.39 ± 0.19 μg/m^3. The values for 3-ethenylpyridine at the low, medium, and high exposures were 0.26 ± 0.39, 39.0 ± 8.7, and 320 ± 40 μg/m^3. Solanesol concentrations ($\pm$SD, $n = 14$) in the low, medium, and high exposures were 1.9 ± 0.36, 22.9 ± 4.4, and 192 ± 18 μg/m^3, respectively.

None of the above analytes could be detected at significant concen-

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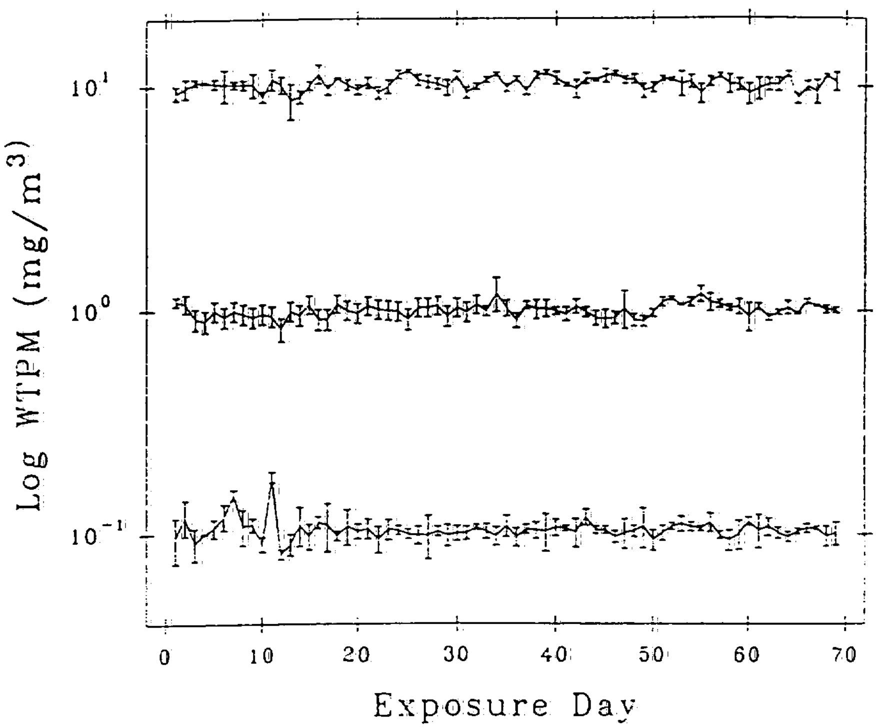

FIGURE 1. Variation in daily concentrations of wet total particulate matter in aged and diluted sidestream smoke presented to experimental animals. Mean ± standard deviations. Targets were 0.1, 1, and 10 mg/m^3.

trations in the sham chamber or in the exposure room. In the exposure room, the mean CO_2 concentration (±SD, n = 15) was 401 ± 50 ppm; in the sham chamber the mean (±SD, n = 14) concentration was 1569 ± 286 ppm. The mean concentrations (±SD, n = 14) in the low, medium, and high chambers were 1430 ± 140, 1702 ± 382, and 1745 ± 257 ppm, respectively.

Particle size distributions at the low, medium, and high exposures were similar, with the average values (±SD, n = 14) being 0.32 ± 0.08, 0.43 ± 0.06, and 0.53 ± 0.03 μm, respectively. The standard geometric deviations were 1.94 ± 0.55, 1.59 ± 0.17, and 1.51 ± 0.11.

In-Life Observations

There were no exposure-related clinical signs or mortalities and the serology results were negative. Animals in the smoke-exposed groups

showed body weights that were not different from those in the sham group (Fig. 2); at no point did animals lose weight (Fig. 3).

Blood COHb concentrations at the end of the exposures were negligible in the sham and low-exposure groups. The means for the medium and high groups ($\pm$SD, $n = 30$) were 0.4 ± 0.4 and $4.9 \pm 0.9\%$, respectively.

The sham and low-exposure groups had plasma nicotine and cotinine concentrations at or below the limit of detection. The mean plasma nicotine for the mid- and high-exposure groups ($\pm$SD, $n = 30$) were 20.5 ± 5.1 and 62.8 ± 9.8 ng/ml, respectively. The mean cotinine concentrations ($\pm$SD, $n = 29$) were 26.6 ± 2.3 and 165 ± 41 ng/ml, respectively.

Necropsy Data

The mean terminal body weight ($\pm$SD, $n = 10$) for sham, low, medium, and high exposures at the 4-day necropsy were 243 ± 10.9, 254.9 ± 10.1, 243.5 ± 9.3, and 247.6 ± 10.8 g, respectively. The mean

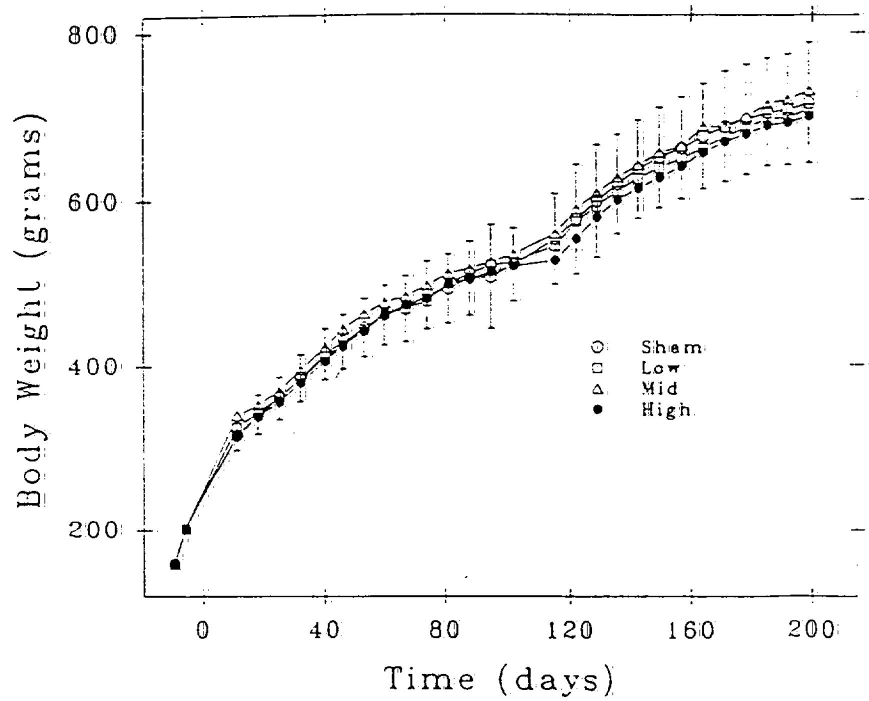

FIGURE 2. Change in body weight of animals exposed to aged and diluted sidestream smoke. Means ± standard deviations.

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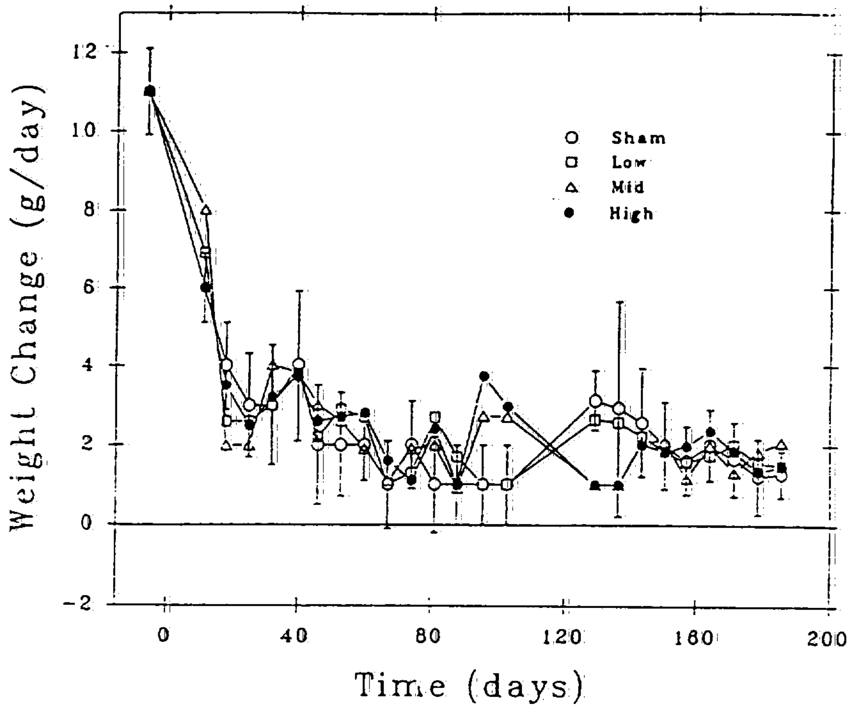

FIGURE 3. Rate of changes in body weight in animals exposed to aged and diluted sidestream smoke. Means ± standard deviations.

terminal body weight (±SD, n = 15–20) for sham, low, medium, and high exposures at the 28-day necropsy were 433 ± 31.8, 428.6 ± 33.5, 447 ± 43.7, and 441.4 ± 35.9 g, respectively.

The mean terminal body weight (±SD, n = 25) for sham, low, medium, and high exposures at the 90-day necropsy were 493.2 ± 38.1, 519.3 ± 41.3, 545.9 ± 58.5, and 508.4 ± 42.8 g, respectively. The mean value for the medium exposure group was 10% greater than that for the sham ($p < .01$), and 5% ($p < .05$) and 7% ($p < .01$) higher than the means for the low and high groups, respectively. At the reversibility necropsy, the means (±SD, n = 12–21) were 738.7 ± 81.9, 712.6 ± 68.9, 734.2 ± 87.9, and 704.7 ± 106.7 g for the sham, low, mid, and high groups, respectively. There were no significant group differences at the reversibility necropsy.

There were no significant differences between the groups for any clinical pathology parameters at any of the necropsies.

There were no exposure-related gross observations at any of the necropsies, nor were there any differences in organ weights.

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Histopathology

In the nasal I section (transverse, immediately caudal to the incisor teeth, Fig. 4), there was minimal to mild epithelial hyperplasia, in the high-exposure group only. In this change, there was hypercellularity and thickening of the respiratory epithelium of the dorsal nasal conchae (nasoturbinates: Figs. 5 and 6) and the adjacent wall of the middle meatus. Figures 7–9 show the distributions of the different severities of this change at the 4-, 28-, and 90-day necropsies, respectively. At each of these necropsies the distribution of the change in the high-exposure group was significantly different from that in the other groups; no such effect was noted at the low or medium exposures.

There were no other histopathological changes noted in animals killed at any of the necropsies, nor were any changes noted in the animals killed at the reversibility necropsy (i.e., the minimal changes noted at the end of the exposure were totally reversible).

The above results were made by a pathologist (JWS) with knowledge of the exposure group. The slides and scores from these readings were reviewed by a second pathologist (Dr. D. L. Dungworth) without knowledge of the exposure.

Because of the very similar incidences and severities seen in the high-exposure groups at the 4-, 28-, and 90-day necropsies, and because these latter results were also similar to those noted in the earlier 14-day study (Coggins et al., 1992), "blind" rereads on a combined set of slides for the high-exposure group only were performed. In the blind rereads, the pathologists (operating independently) thus had knowledge of the exposure group, but not of the duration of the exposure. Only the male animals were used from the 14-day study, mixed with the 4-, 28-, and 90-day slides of the present study. The unmixed data from one of the pathologists (JWS) are presented as Fig. 10: there were similar responses at each of the time points, indicating no progression of the hyperplasia with continued exposure.

DISCUSSION

The inhalation data presented here show that the target concentrations were met and that the aerosols presented to the animals were respirable by them. As in the earlier work (Coggins et al., 1992), we have no explanation for the low concentrations of both nicotine and 3-ethenylpyridine in the low chamber, although concentrations were above those noted in the sham chamber. Inhalation of presented aerosols was confirmed in the medium and high groups through blood COHb and plasma nicotine and cotinine measurements. Consequently, comparisons can validly be made of the histopathological changes in the different groups. The three biological markers of dosimetry could not

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FIGURE 4. Low-power view of a transverse section of the rat nasal passages, immediately caudal to the incisor teeth. The arrow marks the position of the change shown in Figs. 5 and 6. H&E, original magnification ×45.

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FIGURE 5. High-power view of the tip of a nasal concha (nasoturbinate) from a typical sham-exposed rat. The epithelium is 2-3 cell layers thick. H&E; original magnification ×500.

confirm exposure in the low group, where ADSS concentrations were considered to be at or near "real-world" (Guerin et al., 1992). It is thus very unlikely that these markers could be considered as being acceptable for characterizing human exposures to ETS.

The increased terminal body weights in the medium exposure group at the 90-day necropsy are considered to be a statistical anomaly and are not considered to be biologically significant.

The histopathology results obtained here at 90 days are in agreement with earlier work (von Meyerinck et al., 1989), where only a single concentration of ADSS was used (4 mg/m^3) for 13 weeks at 10 hr/day. In the earlier work, the main histopathological change noted was also in the

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FIGURE 6. High-power view of the tip of a nasal concha (nasoturbinate) from a typical high-exposure rat. The epithelium is 5–6 cell layers thick (epithelial hyperplasia). H&E, original magnification × 500.

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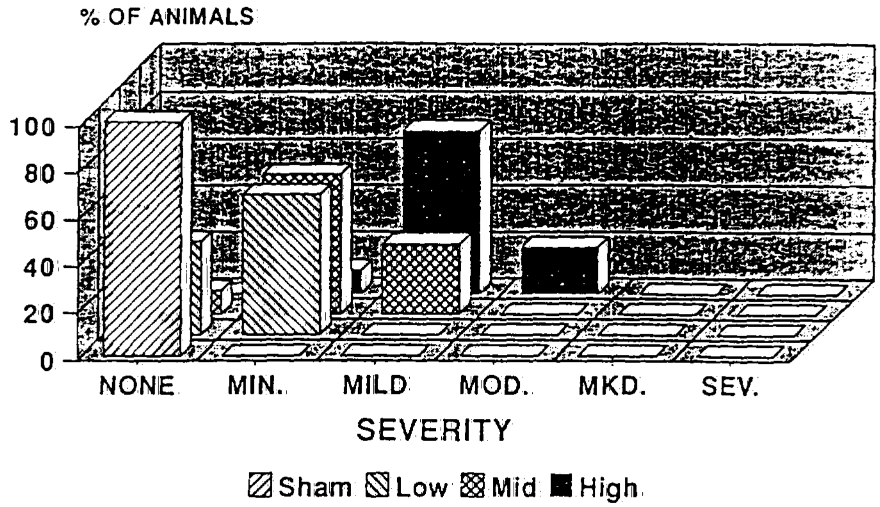

FIGURE 7. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 4-day necropsy of animals exposed to aged and diluted sidestream smoke. MIN, minimal change; MILD, mild change; MOD, moderate change; MKD, marked change; SEV, severe change.

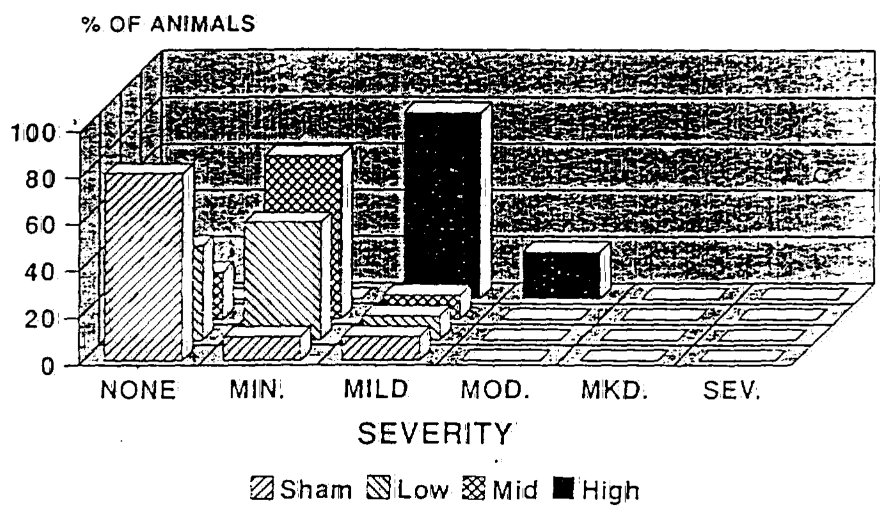

FIGURE 8. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 28-day necropsy of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

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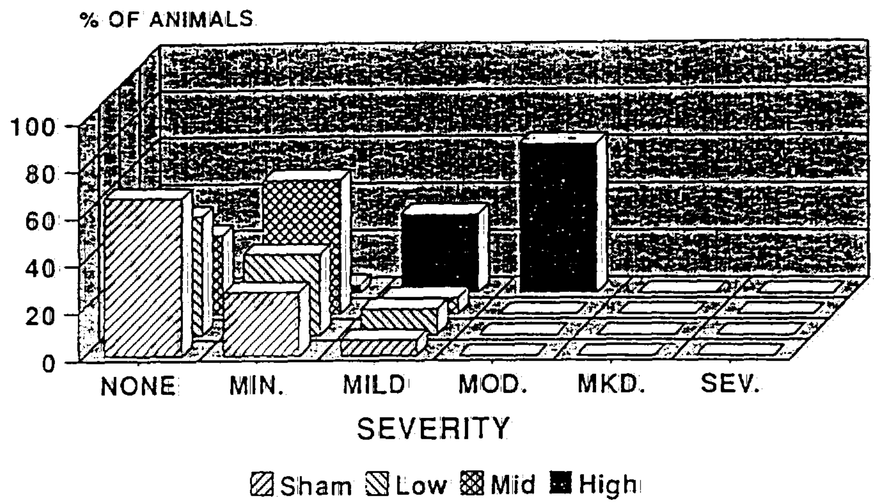

FIGURE 9. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 90-day necropsy of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

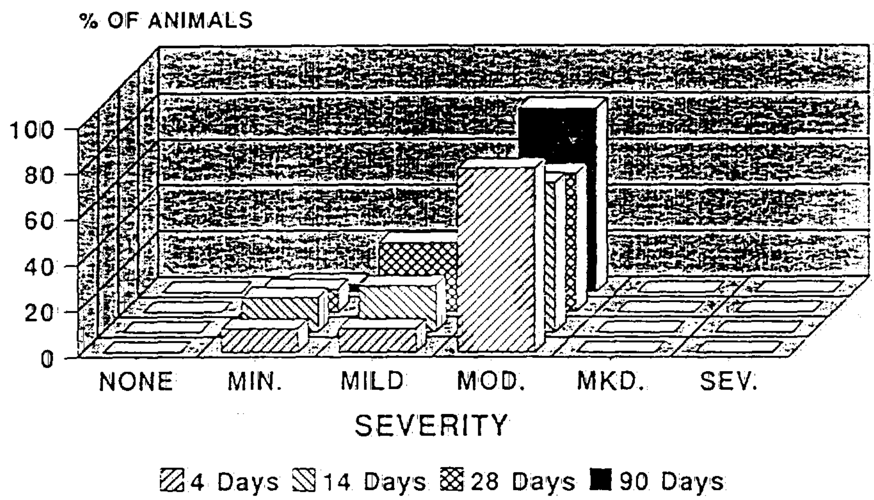

FIGURE 10. Distribution of epithelial hyperplasia in the rostral nasoturbinates in the high-exposure groups at the 4-, 28-, 14-, and 90-day necropsies of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

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rostral nasal cavity of both rats and hamsters—a change that appears morphologically to be very similar to that noted in the high-exposure group in the present study. The results presented here at 4, 28, and 90 days of exposure are similar and are also in agreement with the data presented in a 14-day study using the same concentrations and daily durations (Coggins et al., 1992). This finding suggests a complete lack of progression of the histopathology change with continued exposure. In this and the Coggins et al. (1992) study the histopathology changes noted were completely reversible once the exposures had been stopped. For these reasons, the epithelial hyperplasia in the nasoturbinates is considered to be an adaptive and reactive response to repeated irritation.

The exposure levels used in this study were carefully chosen with respect to typical concentrations in real-life environments. The low exposure used is typical of exposure concentrations of particulate matter in public places where smoking is allowed without restriction, although the contribution of tobacco smoke to such exposures has been shown to be less than 50% (Guerin et al., 1992). In the present work, the contribution of tobacco smoke to the aerosol presented to the animals was clearly 100%. The exaggerations of field values should then in reality be 2-, 20-, and 200-fold rather than 1-, 10-, and 100-fold. Results of a recent survey (Turner et al., 1992) showed an overall mean value of 46 $\mu g/m^3$ for respirable suspended particulates in areas where smoking was allowed, suggesting that the figure of 200 for the exaggeration of field exposures may be an underestimate.

The results again show only a minimal effect of exposure to very high concentrations of ADSS, the only effect being the completely reversible and nonprogressive changes seen in the rostral nasal cavity at the high exposure only. Even with the extended durations (5 times those of the 14-day study) no changes were reported in any organ other than the rostral nasal cavity, and even here the change was described as being very mild. No histopathological findings of any kind were noted at the medium or low exposures. Since the concentrations of smoke used were gross exaggerations (at least 100-fold) of any reasonable field situation for ETS (Guerin et al., 1992), we conclude that ETS is unlikely to have any significant toxicological activity in humans. The NOEL for the 90-day study, based again on nasal histopathology, is at least 1 mg/m^3.

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Ninety-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette: DNA Adducts and Alveolar Macrophage Cytogenetics

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Ninety-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette: DNA Adducts and Alveolar Macrophage Cytogenetics, LEE, C. K., BROWN, B. G., REED, E. A., COGGINS, C. R. E., DOOLITTLE, D. J., AND HAYES, A. W. (1993). *Fundam. Appl. Toxicol.* 20, 393–401.

To study the genotoxic effects of subchronic exposure to environmental tobacco smoke, Sprague–Dawley rats were exposed to 0, 0.1, 1.0, and 10 mg total particulate matter (TPM)/m^3 of aged and diluted sidestream smoke (ADSS) from 1R4F reference cigarettes 6 hr per day, 5 days a week for 13 weeks. DNA from lung, heart, larynx, bladder, and liver was tested for adduct formation by the ^{32}P-postlabeling assay after 28 (except bladder) and 90 days of exposure and 90 days after cessation of exposure. In addition, alveolar macrophages from animals exposed for 28 or 90 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any tissue in any of the animals exposed to 0.1 or 1.0 mg TPM/m^3. However, increased levels of DNA adducts with diagonal radioactive zones were observed in lung, heart, and larynx DNA of animals exposed to the highest concentration of ADSS (10 mg TPM/m^3). Adduct analyses with varying amounts of DNA from lungs of mid- and high-exposure animals clearly indicate that the dose–response for DNA adduct formation is nonlinear. The adduct levels were highest after 90 days of exposure and were significantly reduced in all target tissues 90 days after cessation of exposure. Chromosomal aberrations in alveolar macrophages were not elevated in any group after 28 or 90 days of exposure. These results indicate a no-observed-effect-level (NOEL) of at least 1.0 mg/m^3 for DNA adduct formation in lung, heart, and larynx, and a NOEL of at least 10 mg/m^3 for the induction of chromosome aberrations in alveolar macrophages, under the conditions of this study. © 1993 Society of Toxicology.

The potential health consequences of exposure to environmental tobacco smoke (ETS) are complex issues which concern both scientists and the general public (Department of Health and Human Services, 1986; National Research Council, 1986). Epidemiological studies intended to address the effects of exposure to ETS have reported both positive and negative associations between lung cancer and exposure to ETS (Department of Health and Human Services, 1986; Layard, 1986). Several *in vitro* studies conducted on highly concentrated extracts of ETS have reported positive results, indicating that it is genotoxic under these study conditions (Claxton *et al.*, 1989; Lewtas *et al.*, 1987; Ling *et al.*, 1987; Lofroth *et al.*, 1988; Lofroth and Lazardis, 1986). However, the results of these *in vitro* studies are of limited value for the evaluation of the effects of exposure to ambient concentrations of ETS. Genotoxic effects of exposure to ETS can be best determined by controlled experiments in which animals are exposed to known concentrations of ETS followed by measurement of sensitive biological markers for genotoxic damage.

ETS consists of aged and diluted sidestream smoke (ADSS, 85%) mixed with aged and diluted exhaled mainstream smoke (15%). Studies (Guerin *et al.*, 1992; Turner *et al.*, 1992) have shown that the concentration of respirable suspended particles (RSP) in offices and restaurants where smoking is unrestricted is approximately 0.1 mg/m^3, rarely exceeding a concentration of 0.3 mg/m^3. Less than 50% of the RSP originated from cigarette smoking (Guerin *et al.*, 1992). However, studies on the biological responses to cigarette smoke exposure at or near these concentrations are scarce (von Myerinck *et al.*, 1989). In our previous study (Lee *et al.*, 1992), rats were exposed to ADSS as a surrogate for ETS for 14 consecutive days. No increase in DNA adducts was observed in animals exposed to ambient (0.1 mg/m^3) and 10-fold exaggerations (1 mg/m^3) of ETS. Adducts were increased in lung and heart but only at 10 mg/m^3, the 100-fold exaggerated level of the ambient ETS concentration. Cytogenetic analysis in pulmonary alveolar macrophages was completely negative at all ADSS concentrations (Lee *et al.*, 1992).

In the present study, we have significantly extended both the length of the ADSS exposure and the length of the post-exposure recovery period. Rats were exposed to ADSS at

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concentrations of 0.1, 1.0, and 10 mg/m^3 for 90 days followed by 90 days of recovery. Two sensitive biological markers for genotoxicity, chromosomal aberrations in pulmonary alveolar macrophages (PAM) and DNA adduct formation in major target and nontarget organs, were analyzed after 28 and 90 days and after the recovery period. The results of the 90-day study confirm the existence of a no-observed-effect-level (NOEL) of at least 1.0 mg/m^3 for DNA adduct formation and a NOEL of at least 10 mg/m^3 for the induction of chromosomal aberration in PAMs even in the extended exposure duration of 90 days.

MATERIALS AND METHODS

Materials. Calf spleen phosphodiesterase (2 U/mg) was obtained from Boehringer-Mannheim (Indianapolis, IN). Micrococcal nuclease (100–200 U/mg) and nuclease P1 (255 U/mg) were from Sigma (St. Louis, MO). T_4 polynucleotide kinase was from GIBCO-BRL (Gaithersburg, MD), and [γ-^{32}P]ATP (3000 Ci/mmol) was from New England Nuclear (Wilmington, DE). Polyethyleneimine cellulose (PEI-cellulose) coated thin-layer chromatographic (TLC) sheets (Machery Nagel) were purchased from Brinkmann Scientific (Westbury, NY). The 1R4F reference cigarettes (0.8 mg nicotine and 11.5 mg tar per cigarette) were obtained from the Tobacco and Health Research Institute, Lexington, KY.

Experimental animals. Details on experimental animals have been reported elsewhere (Coggins *et al.*, 1993). Briefly, 5-week-old male Sprague–Dawley rats (Charles River, Raleigh, NC) were acclimated for 2 weeks prior to exposure in 2-M^3 stainless steel inhalation chambers. The animal rooms had controlled lighting (12 hr dark and 12 hr light), temperature (20–24°C), and humidity (40–60% relative humidity). Animals were allowed unrestricted access to feed (Purina Rodent Chow 5002) and distilled water, except during the smoke exposures. The animals were housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Subpart F.

Smoke generation and exposure conditions. Smoke generation and exposure conditions have been described previously (Coggins *et al.*, 1993). Briefly, smoke was generated from 1R4F research cigarettes with a 30-port AMESA generator (CH Technologies, Westwood, NJ) fitted with an aluminum cone for collection of sidestream smoke. Cigarettes were smoked according to the Federal Trade Commission method (a 35-ml puff of 2 sec duration, once per minute) except that instead of a fixed butt length, a fixed number of puffs (7) was taken (Baumgartner and Coggins, 1980; Ayres *et al.*, 1990). Mainstream smoke was exhausted and discarded and the sidestream smoke was drawn into a common plenum. Different amounts of aged and diluted sidestream smoke were provided for each chamber and mixed with dilution air drawn from the animal room through HEPA filters. Target concentrations for suspended particulates were 0, 0.1, 1, and 10 mg/m^3. Exposures were for 6 hr per day, 5 days a week for 13 weeks (total of 65 exposures). Dermal absorption and oral ingestion of particulates after preening were minimized by the use of nose-only restraint tubes inside the chambers.

Experimental design. Rats were divided into four exposure groups: sham, low (0.1 mg TPM/m^3), medium (1.0 mg TPM/m^3), and high (10.0 mg TPM/m^3) exposures. For DNA adduct analysis, each group contained 15 male animals. Five animals in each group were killed after 28 and 90 days of exposure for DNA adduct analysis. The remaining 5 animals in each group were kept for a further 90 days without smoke exposure for the reversibility study. For chromosomal aberrations in PAM, 5 animals in each exposure group were killed after 28 and 90 days of exposure. No reversibility study was conducted for chromosomal aberrations in PAM.

DNA isolation. DNA was isolated and purified as described previously (Lee *et al.*, 1992). Briefly, whole lung, heart, liver, larynx, and bladder samples were homogenized, centrifuged, and the supernatant extracts discarded. Pellets were resuspended and DNA was isolated on a Genepure 341 nucleic acid purification system (Applied Biosystem, Inc., Foster City, CA) by solvent extraction and enzymatic digestion of protein and RNA. The DNA concentration was estimated spectrophotometrically prior to analysis (1 A_{260} = 50 μg DNA/ml). Absorbance ratios (260/280) of all DNA samples ranged from 1.6 to 1.8.

^{32}P-postlabeling assay. Five micrograms of DNA was digested to 3′-nucleotides by incubation at 37°C for 3.5 hr in a total volume of 10 μl containing 0.6 U of micrococcal nuclease and 5.0 μg of spleen phosphodiesterase. Adducted nucleotides were enriched by the nuclease P1 procedure (Reddy and Randerath, 1986) or by butanol extraction (Gupta, 1985) and samples were then [5′-^{32}P]phosphorylated at 37°C using 75 μCi of [γ-^{32}P]ATP with a specific activity of ~3000 Ci/mmol and 4.5 U of T_4 polynucleotide kinase (Lee *et al.*, 1992). ATP excess was confirmed by one-dimensional separation of normal nucleotides from unreacted [^{32}P]ATP in PEI-cellulose TLC using 0.28 M ammonium phosphate/0.05 M sodium phosphate, pH 6.6. Resolution of the ^{32}P-labeled adducts was carried out as previously described (Gupta *et al.*, 1982) with 4 μg of DNA applied on PEI-cellulose TLC sheets using the following solvents: D1, 1.0 M sodium phosphate, pH 6.0 (overnight onto a wick); D3, 5.3 M lithium formate, 8.5 M urea, pH 3.5; D4, 1.2 M lithium chloride, 8.5 M urea, 0.5 M Tris base, pH 8.0 (onto a wick); D5, 1.7 M sodium phosphate, pH 6.0 (overnight onto a wick).

Quantification of DNA adducts. The specific activity of each batch of [^{32}P]ATP was determined by measuring the kinase-catalyzed incorporation of radioactivity into 10 pmol of 2′-deoxyadenosine 3′-monophosphate (dAp) (Reddy and Randerath, 1986). Values of specific activity fell within the range of 1.5 to 2.5 × 10^6 cpm/pmol. TLC plates were wrapped in Mylar plastic and scanned for 8 hr using the AMBIS radioanalytic imaging system (AMBIS Systems, Inc., San Diego, CA) under an argon atmosphere. The counting efficiency averaged approximately 20%; a 3.2 × 3.2-mm resolution plate was used. The use of the AMBIS radioanalytic imaging system in two-dimensional TLC and in ^{32}P-postlabeling assay of DNA adducts has been previously reported (Hook *et al.*, 1990; Turteltaub *et al.*, 1990; Lee *et al.*, 1992). The adduct maps comparing the conventional autoradiography and AMBIS scanning are shown in Fig. 1. Relative adduct labeling (RAL) values were calculated by

$$\mathrm{RAL} = \frac{\text{cpm in adduct nucleotides}}{\text{sp act (ATP)} \times \text{pmol dNp applied to TLC}}$$

In vitro modification of DNA with cigarette smoke condensate (CSC). In order to prepare the DNA–CSC adduct to be used as a positive control, 1 mg of lung DNA isolated from Sprague–Dawley rats was treated with 5 mg (in 500 μl of DMSO) of CSC from Kentucky Reference 1R4F cigarettes in the presence of an S9 metabolic activation system (Ames *et al.*, 1975). The S9 concentration was 5% (v/v) and 5.0 ml of the S9 mix was added to the reaction tube. The reaction mixture was incubated for 5 hr at 37°C. DNA was extracted with the ABI Genepure Extractor.

In vivo modification of DNA with benzo[a]pyrene (B[a]P). Female B6C3F1 mice were given a single ip dose of 40 or 60 mg/kg of B[a]P in 0.1 ml of DMSO/corn oil (50/50; v/v). Livers were collected 43 hr after B[a]P administration. DNA was extracted by the Genepure Extractor and used as a source of standard DNA–B[a]P adducts.

Pulmonary alveolar macrophage isolation and analysis of chromosomal aberration. Designated animals were injected ip with colchicine (6 mg/kg, 12 mg colchicine/ml Hank's balanced salt solution (HBSS) stock solution) at the end of the 28- and 90-day time points in the study. Four hours later animals were killed by asphyxiation with 70% carbon dioxide (CO_2) and the tracheas were cannulated with 14G iv catheters. The lungs and

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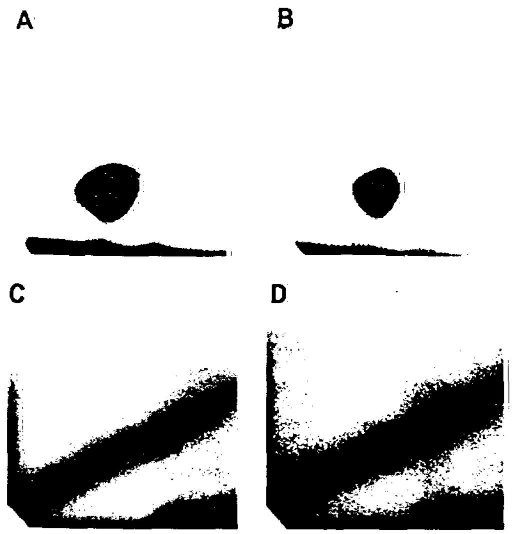

FIG. 1. TLC maps of B(a)P–DNA and CSC–DNA adducts developed by AMBIS scanning and autoradiography. B(a)P–DNA adducts (A, B) were prepared from liver of B6C3F1 mouse injected ip with 40 mg/kg of B(a)P and assayed by P1 nuclease enhancement. CSC–DNA adducts (C, D) were prepared *in vitro* as described under Materials and Methods. The adduct assays were done with 1.6 μg of B(a)P-modified DNA and 4 μg of CSC-modified DNA. TLCs were scanned 2 and 10 hr with AMBIS radioanalytic imaging system for B(a)P–DNA (A) and CSC–DNA (C), respectively. For autoradiography, Kodak XAR-5 diagnostic films were exposed for 7 and 24 hr for B(a)P–DNA (B) and CSC–DNA (D), respectively, at −80°C.

heart were removed and placed in ice-cold HBSS (Ca^{2+} and Mg^{2+} free) for 30 min. The lungs were lavaged and massaged repeatedly using a 10-ml syringe filled with 7 ml of ice-cold HBSS. The lavage fluid was collected in a 50-ml centrifuge tube on ice. The lavage fluid was centrifuged and the supernatant fluid drawn off. The pellet was resuspended in prewarmed (37°C) 75 mM KCl and incubated at 37°C for 25 min. One milliliter of modified Carnoy's (6:1 absolute methanol/glacial acetic acid, v/v) was added to the tubes before centrifugation. After centrifugation the cells were fixed first with modified Carnoy's and then three more times with Carnoy's fixative (3:1). Metaphase spreads were prepared. Slides were then stained for 5 min with Giemsa diluted to 1:20. Fifty metaphases for each animal were scored for chromosome aberrations. Scoring was done without knowledge of treatment group.

Statistical analysis. For DNA adduct data, Levene's test for equality of variance was conducted. Since unequal variances were found, data were log-transformed. Statistical significance ($p < 0.05$) was evaluated by analysis of variance followed by pairwise student's t tests with Bonferroni adjustment. Fisher's exact test was used to determine the level of statistical significance for chromosomal aberration data.

RESULTS

Inhalation of the ADSS by the rats was confirmed by measurement of blood carboxyhemoglobin and plasma nicotine/cotinine (Coggins *et al.*, 1993). No overt signs of toxicity were observed and no significant body weight differences were noted between any of the exposed groups and the sham controls. All lungs, including the ones from the high-exposure group, appeared normal at necropsy. The only histopathological changes observed were mild hyperplasia of the epithelium of the nasoturbinates, in one section of the nose, in the high-exposure group only. These histopathological changes did not progress with increased exposure and they were reversed after cessation of ADSS exposure (Coggins *et al.*, 1992, 1993).

After 28 days of exposure, adduct levels in all the organs tested (lung, heart, larynx, liver) in animals from low- and medium-exposure groups were similar to those of sham control animals (adduct maps not shown). A few discrete adduct spots, thought to represent endogenous DNA adducts, were visible in both groups and the sham controls. Significantly elevated levels of DNA adducts (Fig. 4) were observed in lung, heart, and larynx DNA of animals from the high-exposure group (10 mg/m^3). ^{32}P maps (not shown) of these DNA samples exhibited a few discrete adduct spots, more intense than found in the sham and low- and medium-exposure groups, and diffuse diagonal radioactive zones (DRZ) extending from close to the origin to the middle right-hand margin of the TLC sheet. The DRZ, presumably consisting of numerous incompletely resolved DNA adducts, appears to be characteristic of DNA adducts observed after tobacco smoke exposure (Randerath *et al.*, 1988). DRZs were observed in the adduct maps of CSC–DNA adduct prepared *in vitro* but not in the benzo[*a*]pyrene–DNA adduct as shown in Fig. 1.

After 90 days of exposure, the same organs cited above, as well as bladder, were examined for DNA adducts. Again, all organs from the low- and medium-exposure groups had levels of DNA adducts which were not visually or statistically different from those of sham controls. Doubling the amount of DNA used for TLC development to 8 μg did not produce any exposure-related adducts (i.e., a DRZ) in the low- and medium-exposure groups (data not shown). Lung, heart, and larynx DNA from the high-exposure group exhibited elevated levels of DNA adducts with DRZ and a few discrete spots. Some of the discrete spots appeared in all exposure and sham groups. The RAL values of total adducts were higher than those in the same organs at 28 days. The liver and bladder DNA, however, remained negative even at the high exposure. Representative adduct maps of lung, heart, and larynx tissues from 90-day necropsies are shown in Fig. 2. The scanning time for these samples was 8 hr. Increasing scanning times to as long as 30 hr did not reveal any additional adducts (data not shown). In addition to the P1 nuclease method for adduct enrichment, butanol extraction was also used for both lung and heart DNA from the 90-day samples. The results (data not shown) were similar. The butanol method did not reveal any adduct spots or

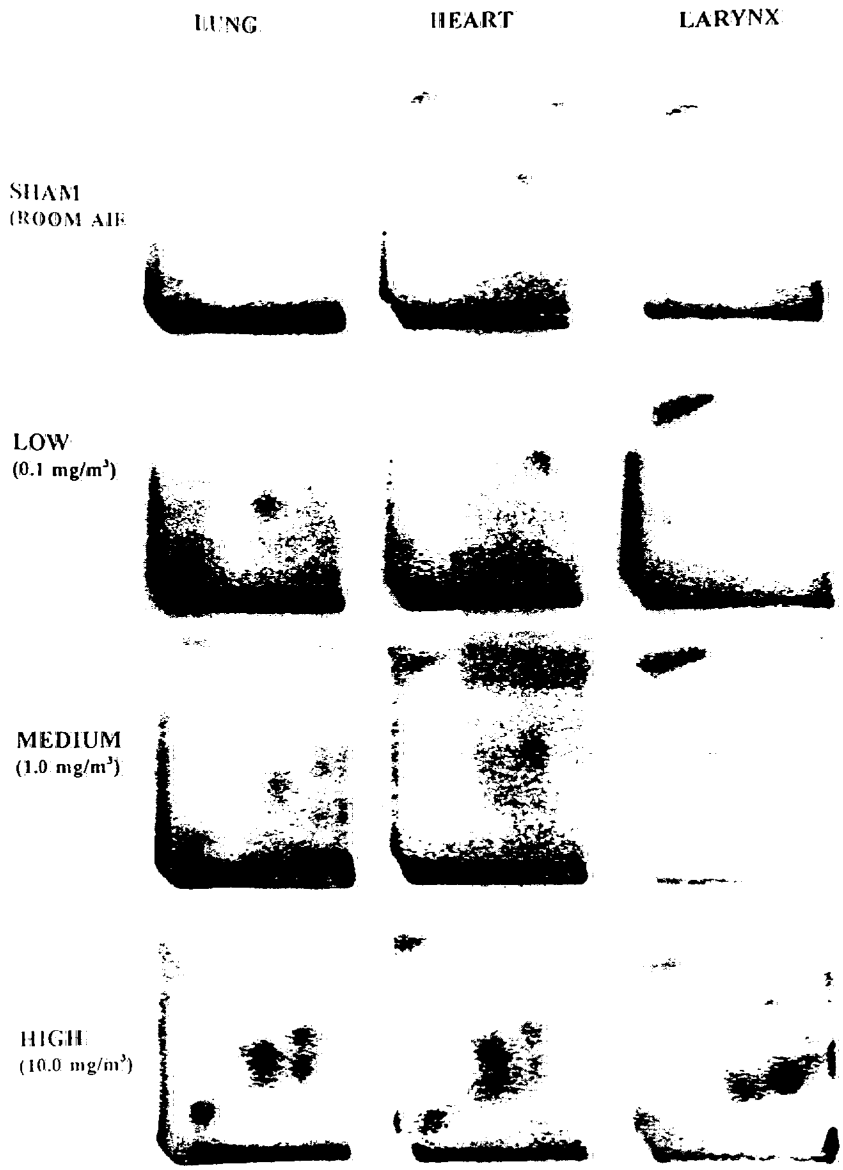

FIG. 2. DNA adduct maps of lung, heart, and larynx tissues of Sprague–Dawley rats exposed to aged and diluted sidestream smoke for 13 weeks. DNA adducts were analyzed using a P1 nuclease version of the ^{32}P-postlabeling assay. 4 μg of DNA were applied on PEI-cellulose TLC for adduct development. TLCs were scanned for 8 hr with AMBIS radioanalytic imaging system.

DRZs which were absent in the nuclease P1 method. Several TLC plates were also scanned using a PhosphorImager instrument (Molecular Dynamics, Sunnyvale, CA) for up to 16 hr. The images developed (not shown) were identical to those obtained using the Ambis after 8 hr of scanning.

The absence of discernible DRZs and statistically nonsignificant levels of total adduct level in the medium- and low-exposure animals could be due to a limit in sensitivity of the ^{32}P-postlabeling assay. To test this possibility, 90-day lung DNA samples from the high-exposure group were postlabeled and an aliquot representing 0.4 μg of DNA was spotted on TLC for adduct resolution. As shown in Fig. 3, the adduct maps of the high-exposure group obtained with 0.4 μg of DNA clearly demonstrated the presence of DRZs and other discrete spots. These results coupled with the absence of DRZs in the 4- and 8-μg samples of DNA from the medium-exposure animals effectively rule out the possibility that the absence of DRZs in the medium-exposure ani-

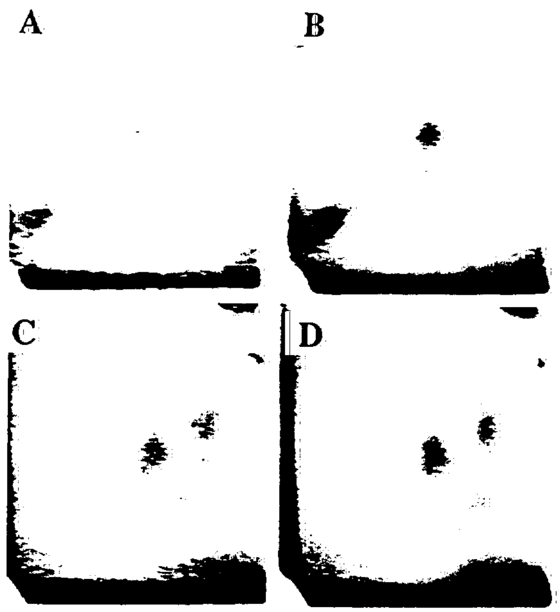

FIG. 3. DNA adduct maps of lung tissues from the high- (A, B) and the medium-(C, D) exposure groups as analyzed with 0.4 and 4 μg of DNA samples, respectively, on TLC. A P1 nuclease version of ^{32}P-postlabeling assay was employed. The TLCs were scanned for 8 (A, C) and 60 hr (B, D) with AMBIS. Note the presence of DRZs in the high-exposure group (A, B), but no corresponding DRZs in the medium-exposure group.

mals is due to the lack of sensitivity of the assay. These data clearly indicate that the dose–response for DNA adduct formation is nonlinear at ADSS concentrations between 1.0 and 10 mg/m^3.

Adduct assays from animals examined 90 days after cessation of ADSS exposure exhibited significant decreases in total adduct levels compared to those before the cessation in the high-exposure group. The RAL values of all the tissue samples (lung, heart, larynx, liver, and bladder) at 28-, 90-, and 180-day necropsies are presented in Fig. 4. It is clear from these results that DNA adducts in low- and medium-exposure groups did not increase with increased duration of exposure; the adducts in lung, heart, and larynx DNA of the high-exposure group exhibited the highest RAL values after 90 days of exposure and then decreased after cessation of ADSS exposure.

The results of the chromosomal aberration assay in PAM after 28 and 90 days of exposure are presented in Table 1. Frequencies of cells with aberrations in all three ADSS-exposed groups are minimal and not different from those of the sham control in both 28- and 90-day samples. Aberrations were mostly chromatid-type rather than chromosome-type in all samples. Animals injected with the positive control, cyclophosphamide, exhibited a statistically significant increase in chromosomally aberrant cells.

DISCUSSION

In the present study, ADSS was used as a surrogate for ETS and animals were exposed subchronically to three concentrations (0.1, 1.0, and 10 mg total particulate matter (TPM)/m^3) of ADSS, representing ambient, 10-fold, and 100-fold exaggerated concentrations of the particulate matter normally found in indoor environments where smoking is occurring (Guerin *et al.*, 1992). When the fact that tobacco smoke accounts for less than 50% of RSP in indoor air (Guerin *et al.*, 1992) is considered, the ADSS concentrations in our study in reality represent 2-, 20-, and 200-fold exaggerations of field values. To evaluate the genotoxic effects of exposure, we chose two sensitive and relevant biomarkers of genotoxicity, chromosomal aberrations in pulmonary alveolar macrophages and DNA adducts in major internal organs as measured by the ^{32}P-postlabeling assay.

The ^{32}P-postlabeling assay has the advantage of high sensitivity (1 adduct in 10^{9-10} nucleotides) as well as not requiring knowledge of the chemical identity of the adducts. Therefore, it is suitable for the study of molecular dosimetry of covalent DNA binding at low concentrations of complex mixtures such as ETS. A dose–response relationship between the DNA adduct levels in the target tissues and application doses of a series of complex mixtures has been reported (Gallagher *et al.*, 1990; Jahnke *et al.*, 1990). The presence of DNA adducts in smokers has been reported, with significantly reduced levels in exsmokers and nonsmokers (Phillips *et al.*, 1988; Randerath *et al.*, 1989; Cuzick *et al.*, 1990; Phillips *et al.*, 1990; Geneste *et al.*, 1991). In rodents, adduct formation has been reported in lungs and other respiratory tissues (Bond *et al.*, 1989; Gupta *et al.*, 1989; Gairola and Gupta, 1991; Reddy and Randerath, 1990) following cigarette smoke exposure.

The discrete DNA adducts detected in the low- and medium-exposure groups were qualitatively and quantitatively similar to those observed in the sham group. Although the identity of these discrete adduct spots seen in all the tissue samples is unknown, they are also present in the high-exposure group. The adduct maps of tissues after exposure to cigarette smoke or other complex mixtures have been shown to include DRZs in addition to these discrete spots. Because of its unique appearance, a DRZ may serve as a "fingerprint" for exposure to cigarette smoke. Clearly visible DRZs were observed in select organs (lung, heart, and larynx) of the high-exposure group only. The discrete spots also increased in their intensity in the high-exposure group, indicating that exposure to ADSS at above 10 mg TPM/m^3 accelerated the preexisting DNA lesions in the rat tissues. Such observations were reported in other smoke exposure studies (Gupta *et al.*, 1989; Gairola and Gupta, 1991). Statistical analysis of the RAL values confirmed that there were more total DNA adducts present in several organs of the high-exposure group compared to those in the

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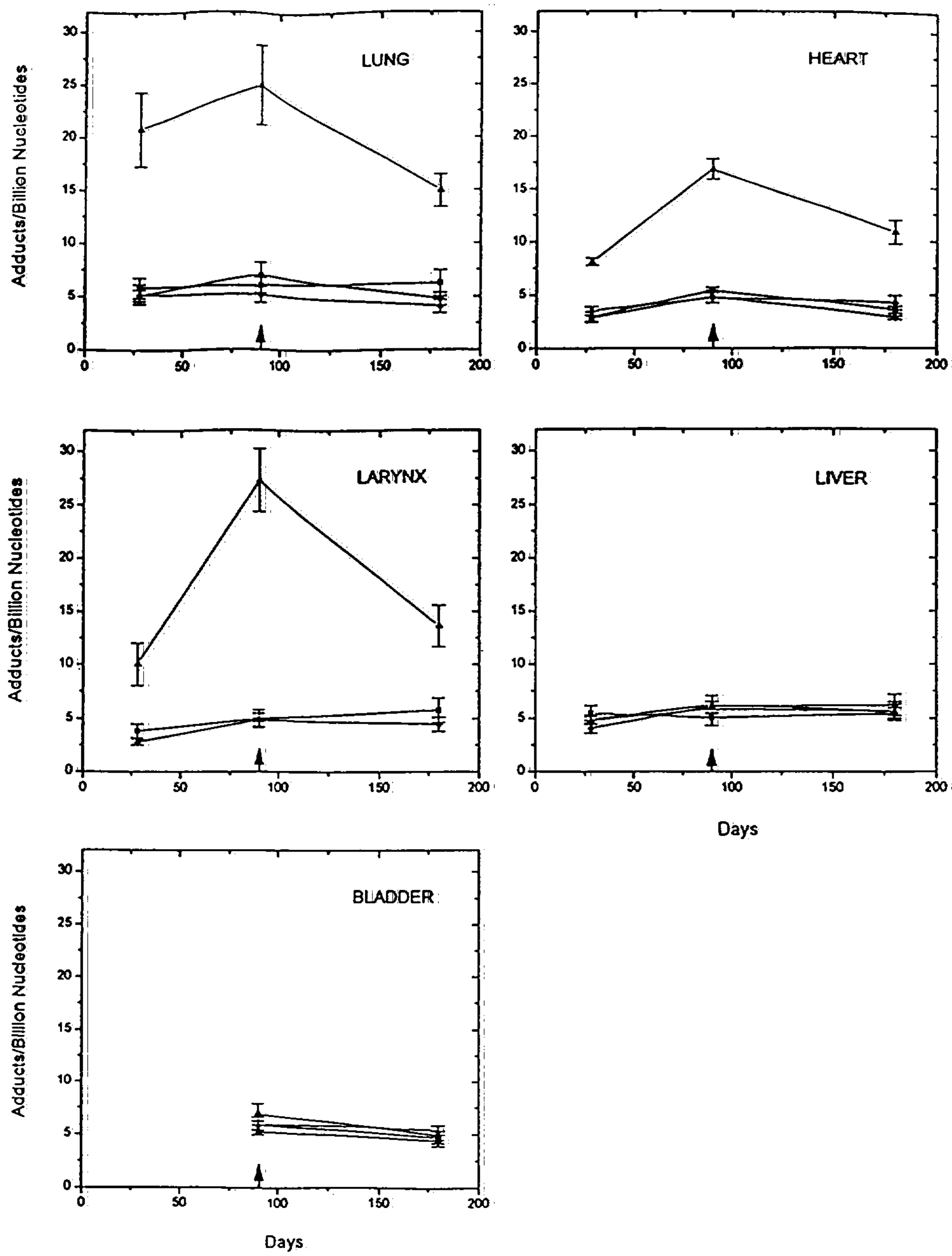

FIG. 4. Time course of adduct formation in the indicated tissues of Sprague–Dawley rats exposed to aged and diluted sidestream smoke. Adducts were determined after 28 (except bladder) and 90 days of ADSS exposure and 90 days after cessation of exposure (180 days). Arrows in the figures indicate 90 days, when the ADSS exposure ended. DNA adducts were analyzed by the P1 nuclease version of ^{32}P-postlabeling assay. Mean RAL values were determined from five animals in each exposure group. The error bars indicate SEM. —Δ— high; —■— medium, —♦— low; —●— sham.

sham and low- and medium-exposure groups. These results were confirmed using both P1 nuclease and butanol extraction methods for adduct enrichment. Bladder and liver did not show ADSS-related adducts even in the high-exposure group. Sprague–Dawley rats exposed to high concentrations of mainstream cigarette smoke for 32 weeks exhibited DNA adducts in lung, heart, trachea, and larynx, but not in bladder and liver (Gairola and Gupta, 1991), qualitatively matching our results with ADSS.

In studies of both humans and laboratory animals, DNA

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TABLE 1

Chromosome Aberration in Pulmonary Alveolar Macrophages of Rats Exposed to Aged and Diluted Sidestream Smoke for 28 or 90 Days

Exposure groups	Number of animals	Number of cells analyzed	Percentage cells with aberration
28 days			
Sham (room air)	5	250	3.2
Low (0.1 mg/m^3)	5	250	2.8
Medium (1.0 mg/m^3)	5	250	2.0
High (10.0 mg/m^3)	5	250	4.0
90 days			
Sham (room air)	5	250	1.2
Low (0.1 mg/m^3)	4[a]	172	0.6
Medium (1.0 mg/m^3)	5	250	0.8
High (10.0 mg/m^3)	5	227	1.8
CP[b] (10 mg/kg)	2	89	13.5*
Saline (0.5 mg/kg)	3	150	0.7

[a] Cell preparation from one animal was lost.

[b] CP, cyclophosphamide (positive control).

* Significantly greater ($p < 0.05$) than sham control by Fisher's exact test.

adducts have been reported to decrease and eventually disappear upon cessation of exposure to genotoxic agents. DNA adducts in rats exposed to diesel exhaust were reduced 4 weeks after the cessation of exposure (Bond *et al.*, 1990). Lung DNA adducts from cigarette smoke-exposed rats were significantly reduced 19 weeks after cessation of exposure (Gupta *et al.*, 1989). In the present study, similar results were observed in that there was a significant decrease in RAL values in the target tissues 90 days after the cessation of exposure.

Most *in vitro* studies with concentrated cigarette smoke condensate have reported positive results in chromosome aberrations and sister chromatid exchange assays. *In vivo* studies with rodents have frequently employed bone marrow cells or lymphocytes to evaluate the clastogenic effects of cigarette smoke exposure by inhalation. In these studies, the evidence of clastogenicity of cigarette smoke exposure in these cell types has not been conclusive. Both positive (Balansky *et al.*, 1987, 1988; Putman *et al.*, 1985; Benedict *et al.*, 1984) and negative (Coggins *et al.*, 1990; Lee *et al.*, 1990; Basler, 1982; Korte *et al.*, 1981) results were reported. Recently, Rithidech *et al.*, (1989) exposed rats to approximately 100 to 200 mg TPM/m^3 for 6 hr/day, 5 days/week for 22 to 24 days and reported a significant increase in the frequencies of chromosomal aberration in the pulmonary alveolar macrophages of exposed animals. It appears that these cells, which are collected in the respiratory tract, are more sensitive to cigarette smoke or airborne chemicals because they represent direct target tissues in inhalation. The negative results in our study indicate the concentrations of ADSS tested were insufficient to induce chromosomal damage in these cells.

Determination of the shape of the dose–response curve in the low-dose region is a difficult and often unresolved task in toxicology and risk assessment. It has often been estimated by linear extrapolation of the effects from high-dose animals (Lutz, 1990). In the previous 14-day ADSS study (Lee *et al.*, 1992), we observed faint but unmistakable DRZs in the lung DNA of the high-exposure group (10 mg TPM/m^3) after 7 consecutive days of exposure. The animals in the current study were exposed 5 days a week for 13 weeks for a net total exposure of 65 days. If the product of concentration (C) times exposure time (T) is linear, one would expect to see DRZs in the medium-dose animals after 65 days since the $C \times T$ for the two studies are similar (7 days $\times$ 10 mg/m^3 = 70 mg days/m^3, 65 days $\times$ 1 mg/m^3 = 65 mg days/m^3). In the present study, the medium-exposure group (1 mg TPM/m^3) after 90 days (65 exposure days) still did not show DRZs and no RAL values higher than those of the sham group, indicating that a linear $C \times T$ extrapolation from the high-exposure 7-day study does not fit the experimental data. In addition, the clear presence of DRZs in 0.4 μg of lung DNA from the high-exposure group (Fig. 3) combined with the absence of similar adducts in 4 (Figs. 2 and 3) and 8 μg of lung DNA from the medium-exposure group provide a strong indication that the adducts in the medium-exposure group, if they exist, are less than one-tenth or one-twentieth of those formed in the high-exposure group. These data clearly support a nonlinear dose–response of DNA adduct formation by ADSS exposures, and indicate a NOEL of at least 1.0 mg/m^3.

The formation of DNA adducts in target tissue is widely regarded as a necessary initial event in the multistage process of chemical carcinogenesis. A nonlinear dose–response in DNA adduct formation with a NOEL could result from a number of host factors such as clearance of inhaled toxicants, detoxification, blocking by nucleophiles, and DNA repair. It is significant that ADSS concentrations (0.1 and 1.0 mg TPM/m^3) which represent above average levels of ETS in most indoor environments where smoking is allowed and a 10- and 20-fold increase in concentration, respectively, did not result in any exposure-related adducts after 90 days of inhalation exposure. None of the concentrations tested significantly increased chromosome aberrations in PAM. Thus, under the conditions of these studies, ADSS at 1.0 mg TPM/m^3 represents a NOEL for DNA adduct formation in lung, heart, and larynx and a NOEL of at least 10 mg/m^3 exists for the induction of chromosome aberrations in alveolar macrophages.

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Histopathological Findings in the Rat and Hamster Respiratory Tract in a 90-Day Inhalation Study Using Fresh Sidestream Smoke of the Standard Reference Cigarette 2R1

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Introduction

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While histopathological changes in the respiratory tract of rats and hamsters in short-term and long-term inhalation studies with *mainstream smoke* (MS) have been reported extensively in literature, there are only three published inhalation studies on rats and rats and hamsters with sidestream smoke in which histopathology is the main end point. In these studies, the findings observed were epithelial hyperplasia and squamous metaplasia in the rostral nose of the rat at a concentration of 4 µg/L (von Meyerinck et al. 1989) and epithelial hyperplasia in the rostral nose of the rat at a concentration of 10 µg/L (Coggins et al. 1992, 1993). No findings were seen in the rat larynx. In the hamster, no histopathological changes were observed in the respiratory tract (von Meyerinck et al. 1989).

Methods and Evaluations

Animals and Housing

Male Sprague Dawley rats, Crl:CDBR (Charles River, Germany), and male Syrian golden hamsters, Lak:LVG(SYR) (Charles River, U.S.A.), were used, the body weight at the start of the inhalation period being approximately 200 and 80 g, respectively. The animals were housed under standardized conditions (room temperature 22 ± 1 °C, relative humidity 55 ± 10%, and light–dark cycle 14.5 hours:9.5 hours) in polycarbonate cages, type 3, with granulated dust-free wood as bedding material. Diet and drinking water were supplied ad libitum except during exposure.

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Animal Exposure

ut: to fresh sidestream smoke (FSS)

The animals were nose-only-exposed for 7 hours/day, 7 days/week for 90 days in glass tubes adapted to the shape of the skull at the front end and sealed with rubber stoppers at the rear end. The TPM concentrations in the FSS of 2 and 6 µg/L are significantly above the levels reached in occupied spaces with smoking (U.S. EPA 1992). The air flow rate in the exposure chamber, cross section 0.1 m × 0.1 m, was 50 L/minute. Sham-exposed animals served as controls.

Group Size

Twenty male rats and 20 male hamsters per group were allocated to one sham exposure group and two FSS groups. Of these, 10 rats and 10 hamsters per group were kept for a 21-day postinhalation period.

Generation of Sidestream Smoke

University of Kentucky standard reference cigarettes 2R1 were smoked on automatic 30-port positive pressure smoking machines (mean puff volume, 35 mL; puffs/cigarette, 9.8; puff frequency/cigarette, 1/minute; puff duration, 2 seconds). The resulting sidestream was collected using a circular hood inside the smoking machine. The maximum age of the smoke was approximately 7 seconds. The two FSS concentrations were obtained by dilution with particle-filtered air.

Characterization of Test Atmosphere

Relevant analytical parameters were determined at appropriate intervals to characterize the FSS and the air used for sham exposure as well as to check the reproducibility of the FSS generation (Table 1).

Carboxyhemoglobin

The steady-state proportion of carboxyhemoglobin was determined at the end of daily exposure three times during the inhalation period to confirm smoke exposure. In the low- and high-FSS groups it was 1.6 and 3.7% for the rats and 2.0 and 4.5% for the hamsters, respectively.

Biological Parameters

The primary parameters were gross pathology and histopathology of the respiratory tract as well as morphometrical determination of the laryngeal epithelial thickness. HE-stained paraffin sections cut at defined levels (Young

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Table 1. Concentrations of relevant analytical parameters.

Analytical parameter	FSS group Low	FSS group High
TPM (µg/L)	2.1	6.0
CO (ppm)	9	22
Nicotine (µg/L)	0.6	1.3
Ammonia (µg/L)	1.1	2.6
Formaldehyde (ppm)	0.19	0.38
Acetaldehyde (ppm)	0.24	0.55
Acrolein (ppm)	0.03	0.07

1981, Lewis 1980, Lamb and Reid 1969) were evaluated semiquantitatively and morphometrically. Secondary parameters were in-life observations, mortality, body weight, and organ weights.

Results

In both species, no smoke-exposure-related effects were seen for in-life observations, mortality, body weight, organ weights, and gross pathology. The histopathological findings observed in rats were as follows:

nose (rostral): reserve cell hyperplasia of the respiratory epithelium

larynx:

- base of epiglottis: hyperplasia of squamous epithelium
- arytenoid projections
  - ventral depression: hyperplasia of cuboidal epithelium floor of the larynx:
  - squamous metaplasia of the pseudostratified epithelium
- vocal cords:
  - lower medial surface: hyperplasia of the squamous epithelium (Figures 1–3)
  - upper medial surface: squamous metaplasia of the pseudostratified epithelium (Figures 4–6)
- vocal folds: hyperplasia of the squamous epithelium

...: finding should ... follow site ...: colon

... to line up ... base and ...ytenoid

The severity of these findings in rats was slight, and they were observed mainly in the high FSS concentration group. No smoke-exposure-related histopathological changes were observed in trachea and lungs. A dose-dependent increase in epithelial thickness in the larynx compared to sham was observed at the floor of the larynx and at the lower medial surface of the vocal cords; the increase at the respective sites was 19 and 32% in the 6 µg/L TPM

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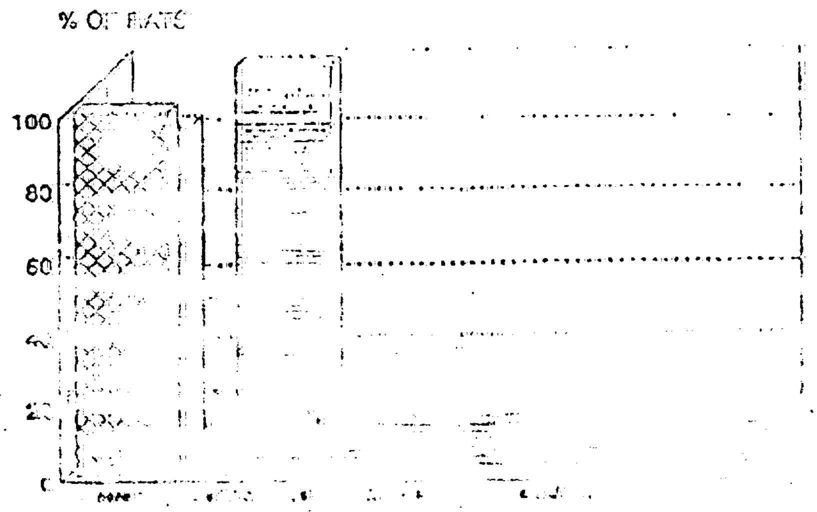

Conclusion

considered to

TPM/L for rats.

magnitude above the average

~~~ Observed Effect Level (NOEL)

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Figure 2. Transverse section at the arytenoid projections, vocal cords, lower medial surface: sham-exposed rat showing normal epithelium; H & E × 147.

Figure 3. Transverse section at the arytenoid projections, vocal cords, lower medial surface: high-dose SS-exposed rat showing hyperplasia of squamous epithelium; H & E × 147.

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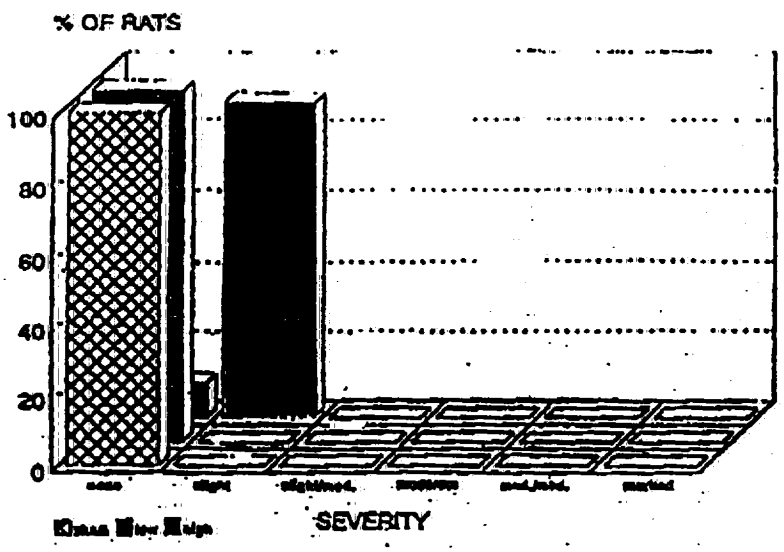

Figure 4. Distribution of squamous metaplasia at the arytenoid projections, vocal cords, upper medial surface, at the end of a 90-day inhalation period.

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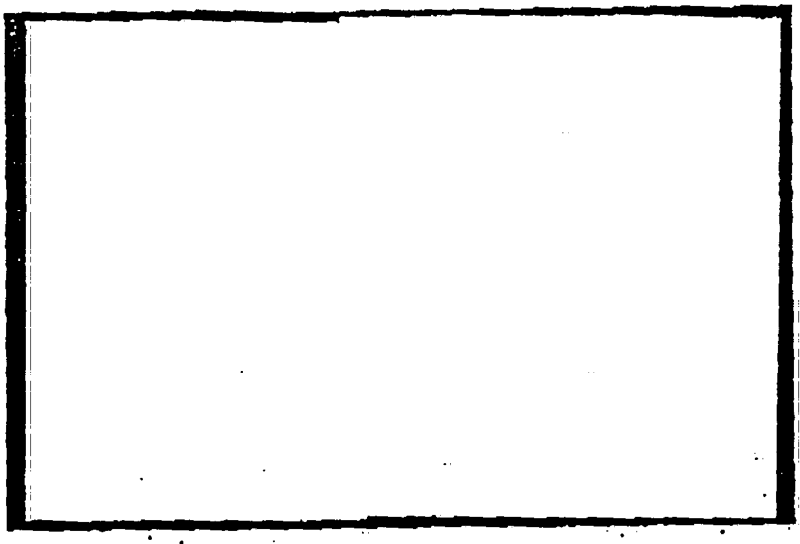

Figure 5. Transverse section at the arytenoid projections, vocal cords, upper medial surface; sham-exposed rat showing normal epithelium; H & E × 147.

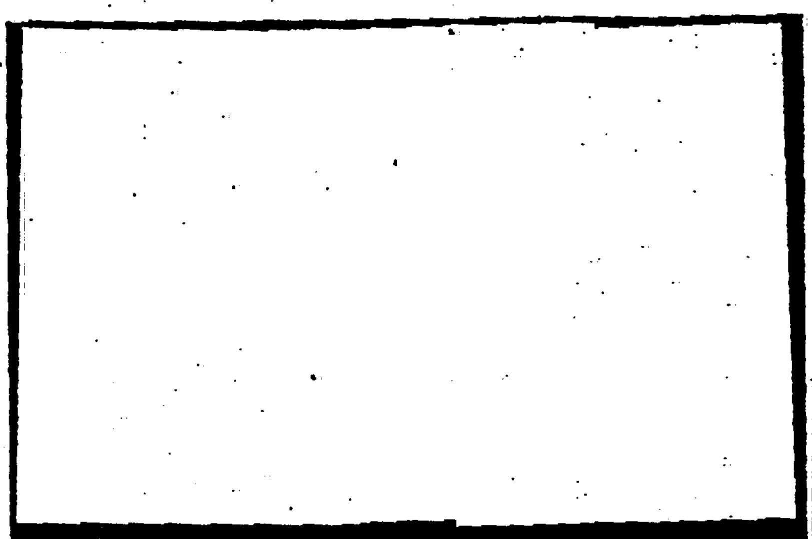

Figure 6. Transverse section at the arytenoid projections, vocal cords, upper medial surface; high-dose SS-exposed rat showing squamous metaplasia; H & E × 147.

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SECTION 6

ETS AND LUNG CANCER

AN OVERVIEW

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SECTION 6

AN OVERVIEW

REFERENCE

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ASSESSING EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE: IS IT VALID TO EXTRAPOLATE FROM ACTIVE SMOKING?

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Abstract

This review examines the question of whether exposure to environmental tobacco smoke (ETS) can be assessed by extrapolation from active smoking. General problems associated with assessing exposure to ETS and the pathophysiological consequences are discussed. Among the topics presented are the dynamic chemical and physical characteristics of ETS and exposure assessment using airborne and biological markers. The reported pathophysiological consequences of ETS exposure are examined in the context of dose and exposure. The conclusion is that it is extremely difficult, if not impossible, to extrapolate from active smoking to ETS exposure with any degree of reliability.

Key words: Environmental tobacco smoke, nicotine, cotinine, adducts, cancer, risk assessment, pathophysiology

Introduction

Tobacco smoke is an exceedingly complex matrix, consisting of several thousand constituents. As it is dispersed in the atmosphere, its chemical and physical complexity can be increased through reactions among its constituents and through evaporation, condensation, coagulation and adsorption or impaction on surfaces [1]. Tobacco smoke as it exists in the ambient environment is termed environmental tobacco smoke (ETS) and is clearly a complex and dynamic material whose properties are influenced by numerous factors. With recent concern that exposure to ETS may present a health hazard to the non-smoker [2,3], a number of risk assessments have been published dealing principally with the possible relationship of ETS to mortality and lung cancer [4]. Among several approaches used for ETS risk assessment has been the comparison between exposure to ETS and active smoking [5–8]. Inherent in such an approach is the assumption that ETS is a dilute form of mainstream smoke (MS) inhaled during active smoking, and that other than the differences in concentration, exposure conditions are similar. Considerable information exists concerning the properties of MS and the conditions of exposure during active smoking [9,10], in large part because material can be collected under reproducible conditions that simulate those to which the smoker is exposed. In contrast, the

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dynamic nature of ETS precludes its characterisation and assessment of exposure to a degree of accuracy possible with mainstream smoke.

Certain criteria must be considered in conducting a risk assessment of a material [11]. Three of these involve consideration of composition and exposure:

1. *A hazard identification*, in which it is determined whether a particular substance is causally linked to specific health effects.
2. *An exposure assessment*, in which the extent humans are exposed to the material has been determined.
3. *A dose-response assessment*, in which the amount of exposure to the material and the probability of occurrence of the specific health effects have been determined. It is the purpose of this article to examine the question of whether exposure to ETS can be assessed by extrapolation from active smoking.

Characterisation of ETS

The following discussion will be concerned with cigarettes only. Mainstream smoke (MS) is that smoke drawn into the mouth through the butt end of the cigarette by the active smoker. Sidestream smoke (SS) is defined as all other smoke emitted from the cigarette with the vast majority being the smoke released from the burning end of the cigarette between puffs [12]. In addition to MS, the active smoker is exposed to SS at levels higher than the non-smoker because of the proximity to its generation. ETS is composed of both SS and exhaled mainstream smoke (EMS), the material exhaled by the active smoker. While it is generally accepted that SS makes a larger contribution to ETS than does EMS, the relative contributions of each material to ETS have not been systematically examined. There is some evidence that EMS contributes little to the gas phase of ETS, however, it does contribute significantly to the particulate phase of ETS [12]. With certain tobaccos, EMS may contribute over 40% of the particles of ETS.

The properties of ETS are influenced significantly by a number of considerations including type of tobacco smoked and smoke density, as well as environmental factors such as dilution, ventilation, temperature, humidity, lighting and adsorption onto surfaces. Additionally, chemical reactions occur changing the composition of ETS; e.g. with time after generation, nitric oxide is converted to nitrogen dioxide [12]. The changes that occur as ETS lingers indoors are termed aging, and contribute significantly to the complexity and dynamic nature of ETS. Because of these factors, it is impossible to provide a definitive chemical and physical description of ETS, its character differing depending on conditions that exist at any given time. As a result, little consistent information exists on the characteristics of ETS under ambient conditions in indoor environments that would allow generalisations about its composition to be made. Because the frequency of puffing and the depth of inhalation differ among smokers, it should be apparent that the relative contributions of SS and EMS to ETS will be different for each ambient environment. Therefore, in addition to environmental factors described previously, the chemical and physical properties of ETS are dependent upon the smoking patterns that occur in an indoor environment. The origins and properties of ETS have been reviewed in detail elsewhere [12].

These considerations notwithstanding, numerous studies have been conducted in an attempt to characterise ETS. These have included the analysis of freshly generated SS, SS allowed to age in controlled environmental chambers, SS allowed to age in well-controlled experimental indoor environments, and ETS in a number of typical indoor environments. Each of these situations has specific limitations as to its usefulness in characterising ETS.

Considerable effort has been directed at characterising freshly generated SS as

a surrogate for ETS, and much data exist on the chemical composition of this material [13–19]. Serious problems are inherent in utilising this approach. First, and most importantly, ETS is much more complex and variable than SS generated in the laboratory due to the presence of undefined proportions of both SS and EMS and the influence of aging on ETS components. Secondly, SS is produced under conditions that do not necessarily represent the smoking pattern of individuals. SS is generated under standardised smoking conditions adopted over 20 years ago in apparatuses that allows it to be rapidly collected for analysis. The conditions are almost always one puff/min of 2 sec duration and a volume of 35ml. Since people smoke with different patterns these conditions do not necessarily simulate those of most smokers [20], and as a result, the quantities of materials released into ambient air will likely vary from those generated using smoking machines. The same objections about standardised smoking conditions could be raised regarding the composition of MS, as well.

The environmental conditions present during generation will influence the levels of chemicals in SS. This is illustrated by the effect of the velocity of air passed over the tip of the burning cigarette when generating SS [21]. In this study, the level of dimethylnitrosamine in SS varied as a function of air flow. Flow rates of 250, 500, 1000, and 1500ml of air/min yielded levels of dimethylnitrosamine of 90, 250, 530, and 680ng/cigarette, respectively. Therefore, depending on the conditions used for generation and collection, values for SS may vary greatly. This is illustrated by the wide range of values reported in the literature for nearly one hundred chemicals reported to be present in SS [15].

Compared to the study of freshly generated SS, utilisation of environmental chambers offers the opportunity to examine the properties under controlled, although not necessarily realistic conditions. The most extensive examination of SS-derived ETS under these conditions appears to have been performed by Eatough and colleagues [14,15,22]. They have utilised an unventilated teflon chamber in studying the properties of ETS originating from SS generated within the chamber. Use of the teflon chamber permits ETS to be studied in a setting where results are not influenced by ventilation or surface properties. Under these conditions, a comprehensive analysis of the chemical composition of the gas and particle phases of ETS was performed, and the behaviour of the particulate phase examined. For example, it was observed that nitrogen dioxide was the major inorganic acid present in the gas phase of ETS, and nicotine, 3-ethenylpyridine, and pyridine were the principal nitrogen bases present. Major particulate phase organic compounds were nicotine, mysomine, solanesol, nicotyrine and cotinine. Greater than 95% of the nicotine was present in the gas phase. As the ETS aged, particles underwent at least three changes. Particles deposited on the wall of the chamber, they coagulated increasing in size, and evaporation from the particles was also significant. The effect of UV radiation was also examined, and it was noted that the level of gas phase nicotine decreased with a concomitant, but less than stoichiometric increase in particulate phase nicotine. An important class of compounds, the nitrosamines were not examined in this system. It will be of considerable interest when the levels and behaviour of the volatile and tobacco-specific nitrosamines are examined under such controlled conditions.

Using a stirred stainless steel chamber to study the properties of ETS, it was reported that smoke particles underwent evaporation over the first few hours [23]. As the ETS aged, particle size increased due to a combination of coagulation and removal of smaller particles by deposition on the surface of the chamber. Similar observations have been made using a ventilated steel chamber [24].

The decay of a number of SS-derived components has also been studied in a

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non-ventilated glass and stainless steel chamber [25]. In particular, polycyclic aromatic hydrocarbons (PAHs) decay at different rates during aging depending on their molecular weights; PAHs below 156 daltons had a longer half-life than those above this value. As with other experimental systems, nicotine decayed more rapidly than particulate material.

Studies have been reported using a modified trailer in which conditions can be controlled with respect to ventilation, temperature, humidity, and circulation [15,22]. Such an environment can be made to simulate closely ambient indoor conditions. Some important observations were made concerning the behaviour of SS-derived ETS in this setting which were similar to those of other workers [12]. For example, the absolute decay of various constituents of ETS was primarily controlled by the rate of ventilation. The rate of decay of nicotine was the most rapid of the components studied, while the NOx–NO species were the most stable.

While controlled chamber studies have provided useful information about ETS, the results must be interpreted with a degree of caution. These conditions only partially simulate the ambient environment in which non-smokers are exposed to ETS. For example, no studies have examined the behaviour of ETS when persons are present in the chamber or when ETS has been generated by smokers so that EMS is also present. In an effort to obtain realistic data on ETS exposure, numerous studies have examined selected chemicals and particles in ETS under a variety of ambient conditions [26–32].

Problems exist in the interpretation of these data, as well. In general, only a few substances have been investigated in each study, with sampling performed over single periods of relatively short time (24 h or less). Such a sampling protocol will fail to describe the daily variations in ETS levels that exist in indoor environments as well as fail to provide a measure of chronic exposure. The lack of specificity of most of the measured substances for ETS (e.g. carbon monoxide and respirable suspended particles) limits conclusions that can be drawn about the composition of ETS in these studies.

Exposure assessment methods and interpretations

It should be clear that there is no defined, reproducibly characterised entity known as ETS, rather it is a constantly changing substance influenced by numerous environmental and personal factors. At present, the published research represents little more than a broad representation of the nature of ETS. Therefore, it is currently not posssible to compare the risks, if any, of exposure to ETS with those reportedly associated with active smoking based on the chemical compositions of each of these materials.

As an alternative solution to the problem of characterising ETS, several approaches have been utilised to assess ETS exposure with the goal of predicting possible related health effects. These efforts have involved the assessment of exposure by use of questionnaires, modelling, surrogate airborne markers, and the assessment of internal dose by use of biological markers (biomarkers).

Reliance on questionnaires alone to assess exposure is fraught with numerous problems including lack of standardisation and validation, responder bias and potential misclassification of subjects. At best their use represents an indirect measure of exposure and cannot provide any quantitative information on specific or total exposure levels or doses of biologically relevant chemicals at target sites [33–36]. Questionnaires can have value when used as part of a more comprehensive exposure assessment. For example, an index of exposure has been developed, which includes questionnaires as one component, along with a daily diary, that correlates well with nicotine collected by a personal monitor [37].

Modelling has been used to assess concentrations of ETS constituents and to estimate exposures [5,6,38]. Data from other studies are normally used in the modelling and, additionally, this approach

requires assumptions which generalise and often oversimplify the exposure conditions.

The use of airborne markers and biomarkers offer the best opportunity to assess exposure to ETS. Unfortunately, reliance on either of these assessments alone for such a complex and dynamic mixture as ETS may result in misleading information. For example, the external dose may not be related to the internal dose as absorption, distribution metabolism and elimination may differ among individual components (particles, water-soluble chemicals, organic materials). The presence of a biomarker in a non-target tissue does not necessarily correlate with the level of a potentially toxic species at the critical cellular site nor whether a disease will result. These limitations in the use of airborne and biological markers are present when applied to exposure assessment for ETS.

Assessment of external exposure

Due to the complex chemical and physical nature of ETS, investigators have relied on tracers, or surrogates, in measuring external exposure to ETS. The National Research Council [2] has provided criteria which should be satisfied in using a surrogate for ETS:

. It should be unique or nearly unique to ETS.
2. It should be present in sufficient quantity that concentrations can be easily detected in air, even at low smoking rates.
3. It should be characterised by similar emission rates for a variety of tobacco products.
4. It should be in a fairly constant ratio to the components of interest under a range of environmental conditions encountered and for a variety of tobacco products.

Unless the first criterion is fulfilled, the remaining criteria are of less significance. To date, no single material has satisfied these criteria.

Respirable suspended particles (RSP) and nicotine have been used most frequently as surrogates for ETS. The use of RSP fails to satisfy the first criterion because of its lack of specificity to ETS. There is a significant level of background RSP not related to ETS in the indoor environment. This has been demonstrated using the property of ultraviolet absorption of RSP as representative of the ETS-specific portion of RSP [29,30]. In several environments where smoking was permitted, it was found that ETS contributed less than 40% of the particles in the indoor environment. If RSP in an indoor environment is to be attributed to ETS, it is necessary to rule out all other sources of RSP. This has not been done satisfactorily in the studies reported to date.

While the measurement of RSP may serve as an index of exposure, it is not a measure of the dose or the amount of particulate material that will be retained in the lungs of those exposed. It is the amount of material retained in the lungs that is believed to have a relationship to health effects, not the amount to which a person is exposed. In fact, the relative retention of ETS particles has never been measured.

Different deposition patterns are to be expected for the particles in ETS and those in MS because of the different breathing patterns of the two population groups. An active smoker inhales MS by mouth often with a deep inhalation followed by a prolonged respiratory pause. Such a manoeuvre increases residence time of particles and gases in the entire respiratory tract, optimising conditions for deposition. In contrast, a non-smoker would inhale ETS principally through the nose using a regular breathing pattern which is much more shallow than that used by active smokers. The shallow breathing pattern would reduce the degree of pulmonary deposition of particulate materials of ETS in non-smokers compared to MS in the active smoker.

Risk assessments for lung cancer have been performed using estimated exposure to RSP from ETS [6,39]. The values used in these calculations were dependent on a number of assumptions that did not consider the limitations of using RSP as a surrogate for ETS. Consequently, the risk values are open to question.

Nicotine has been measured in ambient air using area sampling [40–42] and with personal samplers [37,43,44]. Personal samplers monitor the immediate environment of the subject permitting a more accurate assessment of personal exposure than occurs with area sampling. While airborne nicotine would be specific for ETS, problems exist in using it as a surrogate. Nicotine in ETS is principally in the gas phase [15], while nicotine in MS is almost exclusively in the particulate phase. Therefore, in ETS, nicotine would be serving as a surrogate for gas-phase components only.

Additionally, nicotine in ETS decays more rapidly than other gas-phase components [22], in large part due to its adsorption onto surfaces. It is likely that, once smoking has stopped in a room, the adsorbed nicotine will be slowly released back into the atmosphere. If this occurs, a low level of airborne nicotine may be present in an area where smoking had not occurred for some time giving an inaccurate representation of total ETS exposure.

The ratio of RSP/nicotine has been discussed as a possible monitor for ETS in ambient environments, and in particular as a means for quantifying the ETS-specific RSP [45]. Laboratory studies have given an average ratio of 13.4. Using values for RSP and nicotine from field surveys, it has been concluded that the relationship between these two materials is too variable to use for predictive purposes [46].

The mutagenic properties of RSP have been used to assess exposure to ETS [26]. The principal problem with this approach is the interpretation of the results. The significance of the presence of airborne mutagens has not been established nor have quantitative measures of retention of mutagenic materials been obtained. Because of these uncertainties, the measurement of airborne mutagenicity has provided little information in assessing exposure to ETS.

To date no single material satisfies the criteria as a marker for ETS. Consequently, it has not been possible quantitatively to assess the external dose of ETS a non-smoker receives.

Assessment of internal dose

As an assessment of exposure to ETS, biomarkers can serve as surrogates for the internal dose received. Criteria have been proposed that an effective marker should satisfy [47]:

1. It should be tobacco-specific in order to be certain of its origin.
2. It should have a long half-life so that it serves as an index of exposure over an extended period of time.
3. The marker should give a valid indication of the health risks of exposure.
4. Analytical techniques should be available that can reliably and conveniently measure the low levels of the marker present in non-smokers exposed to ETS.

Biomarkers of ETS exposure have been measured in biological fluids of humans. Several biomarkers have been utilised with varying degrees of success in the assessment of exposure to ETS, including nicotine and cotinine in saliva, blood, and urine, DNA and protein adducts in blood, and mutagenic activity in urine. From these results, investigators have drawn conclusions about exposure, risk of disease, and mortality.

When interpreting studies in which biomarkers have been used to assess exposure or risk, a number of factors must be considered [48]. Data on variation among individuals in absorption, metabolism (including bioactivation and detoxication), kinetics, distribution, excretion, binding to macromolecules and cellular repair must be evaluated. In the

use of biomarkers for assessment of exposure to ETS and assessment of potential health risks, such considerations have not been employed consistently.

Biomarkers such as nicotine, or one of its metabolites, cotinine, in body fluids have been used to assess internal exposure to ETS [41,48–50]. In general, salivary and urinary cotinine provide the best relationship with self-reported exposure to ETS [47,51]. Levels of cotinine in body fluids tend to correlate directly with the number of smokers in the household, the number of hours of exposure, the number of smokers among acquaintances, and are higher in non-smokers married to smokers than in those married to non-smokers.

Nevertheless, significant limitations exist in the use of nicotine or cotinine to assess exposure to ETS. At best, levels of nicotine or cotinine are useful qualitatively to assess exposure. Too many limitations exist for them to be considered quantitative dosimeters from which risk can be estimated [52]. In virtually all studies reported, single samples are taken in the assessment of exposure. Such values are an index of exposure at a specific point in time and do not represent the cumulative exposure that would be required properly to evaluate exposure to ETS. Importantly, the vast majority of nicotine in ETS is in the gas phase while nicotine in MS is predominantly in the particulate phase [[illegible],53]. Therefore, values for nicotine or cotinine in body fluids represent the inhalation of physically different materials in the two exposure groups making their comparative use questionable. Additionally, gas-phase nicotine and particulate-phase nicotine decay at different rates under experimental conditions [22]. Levels of nicotine or cotinine in body fluids provide no information on exposure to other chemicals, particularly those in the particulate phase which are believed to have the most relevance to potential adverse health effects.

It was once thought that one of the attractive features of using nicotine and cotinine as biomarkers was their tobacco specificity. Recent studies indicate that nicotine is not unique to tobacco. A number of vegetables in our diet have been shown to contain nicotine [54,55]. The fact that nicotine, and consequently cotinine, can arise from non-tobacco sources complicates the interpretation of the low-level values of these chemicals that are measured in the body fluids of non-smokers.

Cotinine is only one of a number of metabolites of nicotine and evidence is now indicating that nicotine-n-oxides or trans 3'-OH-cotinine, rather than cotinine, may be the most abundant metabolites of nicotine in the urine [56–58]. Cholerton *et al.*, [56] report a larger coefficient of variation for cotinine than other nicotine-derived metabolites in the urine of smokers. Variations in the metabolic formation of cotinine among non-smokers would further confound the interpretation of cotinine levels.

Complicating this problem even further are pharmacokinetic factors. Nicotine appears to be metabolised at different rates in smokers and non-smokers [59–61]. The half-life of nicotine in plasma appears to be shorter for smokers than for non-smokers, therefore, the relative relationship of values between the two groups will differ depending upon the time of sampling.

Both intralaboratory and interlaboratory variations have been reported for urinary cotinine values [62,63], indicating that comparisons of values among laboratories should be made with caution. Such methodological considerations are of particular significance when values are low as is the case with exposure to ETS.

It seems evident that the measurement of cotinine in body fluids will likely provide misleading information regarding the quantitative exposure to ETS. Considering the factors discussed, a compelling argument can be made against using nicotine or cotinine values for either a quantitative comparison of exposure between smokers and those exposed to ETS or in an

attempt to assess the possible risk of exposure to ETS.

Urinary cotinine as a predictor of health risks of exposure to ETS should be used with caution [64]. Nevertheless, a risk assessment estimating ETS-related mortality has been made using such values. One study reported that urinary nicotine values in non-smokers were 0.7% of the level found in smokers [8] and the assumption made that there are premature deaths from the inhalation of ETS which may be approximately 0.7% of that due to active smoking resulting in 1,000 deaths a year in Great Britain and 4,000 deaths a year in the United States. No consideration was given to the limitations in the use of this marker. Additionally, the authors assumed that the relationship of dose-to-risk is linear between these two exposure extremes, an assumption that has not been shown to be valid. Clearly, this risk assessment is overly simplistic and confounded by a number of significant conceptual problems.

Wigle *et al.* [65], used values for urinary cotinine of active smokers and non-smokers exposed to ETS to assess the relative exposures of non-smokers to components of tobacco smoke that have been reported to be toxic. They concluded that persons exposed to ETS for 20 or more hours per week have exposures to six compounds that have been designated as known or probable human carcinogens which are at least 2% of those of active smokers and, for certain of these compounds, may be more than 20%. In arriving at these estimates, the authors made a number of assumptions which ignore the complexity of the exposure situation. In particular, SS was used as a surrogate for ETS. Such a premise is clearly inappropriate and invalidates any quantitative relationships that might be developed.

DNA and protein adducts have been utilised as biomarkers to assess internal exposure to ETS. Adducts are products derived from covalent reactions between chemicals and biological material such as DNA and proteins. The formation of DNA adducts is reported to be associated with mutagenesis and carcinogenesis [66,67], and adducts are viewed as markers of the biologically effective dose of carcinogens in humans. Recent evaluation of the role of adducts in carcinogenesis indicates that the relationship may not be as direct as initially thought [68].

In spite of the lack of correlation between adduct levels and cancer in a number of studies [69–72] considerable interest continues in their use as molecular dosimeters for carcinogenesis. Although studies have started to examine their possible role in the assessment of exposure to ETS, little useful information currently exists in this context.

For many chemicals, adduct formation following metabolic activation is a necessary, but not sufficient, event to initiate carcinogenesis [73,74]. The formation of adducts may not occur on a region of the genome that is critical in the carcinogenic process. The role of DNA repair must also be considered [75,76]. The variability in repair capabilities in humans [77] will influence the level of adducts present in a tissue. Additionally, genetic polymorphism of drug metabolism in humans has been shown to result in wide inter-individual capacities to activate carcinogens metabolically [78]. Because cancer is a multistage process, and because the level of adducts may be influenced by a multitude of factors including diet [79] it is thought to be unlikely that DNA adducts will provide precise quantitative dosimetry for predicting cancer risk [80], particularly where the level of adducts is as low as observed for ETS exposure. Another line of research in this area involves proteins as target molecules for adduct formation with the goal of serving as a surrogate for DNA adducts [81]. Because of its abundance, haemoglobin has been used to monitor adduct levels associated with exposure to tobacco smoke [82,83].

A potentially attractive aspect of the use of adducts as a dosimeter for ETS exposure, is that they may be useful in

monitoring exposure, at least qualitatively, on a more chronic basis than with other markers. To date, no tobacco-specific adduct has been identified that is capable of fulfilling this goal. Adducts of 4-aminobiphenyl-haemoglobin (4-ABP-Hb) and of benzo[a]pyrene diol epoxide-1-DNA (BPDE-1-DNA) in white blood cells have been compared in smokers and non-smokers [82–84]. While both 4-ABP and benzo[a]pyrene (BP) have been classified as carcinogenic, neither is tobacco-specific. Levels of 4-ABP-Hb adducts have, however, been used to distinguish smokers from non-smokers. The levels of 4-ABP-Hb adducts in non-smokers have been reported to be about one fifth the level found in smokers [82,83]. In one study, BPDE-1-DNA adducts were of little value in distinguishing the two groups [83]. Over a 48 h period, there was little consistency in the presence of adducts in smokers, with many smokers having no detectable levels. Additionally, there was no apparent correlation between the level of 4-ABP-Hb adducts and the level of BPDE-1-DNA adducts in either group.

Adducts of 4-ABP-Hb and 3-ABP-Hb have been measured in the blood of non-smokers with varying degrees of exposure to ETS as assessed by the presence or absence of detectable serum cotinine [82]. In non-smokers exposed to ETS, the 4-ABP-Hb levels were about 40-fold higher than the level of 3-ABP-Hb which in many subjects was below the limit of detection. Due to the lack of a clear cut effect of ETS exposure on 4-ABP-Hb adduct levels, and the inconsistent detectability of 3-ABP-Hb adducts, the usefulness of these markers to discriminate non-smokers exposed to ETS from those who are not exposed, appears questionable.

Recent studies indicate that the turnover of adducts may be more rapid than originally thought, limiting their usefulness to monitor chronic exposure. While the lifespan of haemoglobin is 120 days [81], levels of 4-ABP-Hb adducts in smokers returned to background levels in 6–8 weeks following cessation of smoking [82]. NNK is a tobacco-specific nitrosation product of nicotine that is present in MS and SS and has been classified as carcinogenic in animals [85]. Removal of adducts induced by the injection of NNK, has been examined in rats [69]. Rates of removal of different adducts in target tissues was variable and rapid, occurring within several days. These data indicate that NNK-induced adducts may not be useful as a dosimeter for tobacco smoke exposure.

A very sensitive method for examining the presence of adducts is ^{32}P post-labelling. This technique provides a semi-quantitative estimate of the adduct level in a tissue. At present, there has been little application of this technique in assessing exposure to ETS. In spite of its sensitivity, there are limitations associated with the ^{32}P post-labelling technique. It does not allow identification of the adduct, and basal levels of adducts are reported to increase with age, at least in animals [86]. Using this technique, no increase in DNA adducts was reported in monocytes of non-smokers heavily exposed to ETS [87].

In order to compare the potential risks of exposure to ETS with those reported for active smoking, an extrapolation from high-dose exposure to low-dose exposure is required. DNA adducts have been proposed as a means to do this. The relationship between external dose and biological dose, as assessed by DNA adducts, is dependent on the absorption, distribution, metabolism and excretion of the chemical of interest. The interpretation of biological dose using DNA adducts is influenced by several factors, including the location of the adducts on the genome as well as the mutagenic efficiency of the material, including the base that is modified and the effectiveness of the repair process. Additionally, for certain chemicals, the level of adduct formation is not linearly related to the dose administered. From the existing data, no absolute information is available relating the presence of adducts to a quantitative or qualitative assessment of exposure to ETS.

As a measure of exposure to ETS, studies have been conducted on the capability of concentrated extracts from the urine of non-smokers and persons exposed to ETS to induce mutations in bacteria [88–94]. The rationale behind this approach is that the presence of mutagens in the urine may be an indication that the person has been exposed to chemicals that can ultimately induce cancer. Compared to the mutagenicity of the urine of smokers, activity in the urine of those exposed to ETS is quite low, variable and not always above background levels.

A number of problems exist with the studies attempting to relate urinary mutagenicity to ETS exposure. The experimental conditions of exposure to ETS have often been unrealistic in comparison to that occurring in the ambient environment. Methodological differences exist among studies possibly contributing to some of the inconsistencies. The studies have not always been controlled for the presence of dietary mutagens, an important confounding factor [95]. Importantly, the putative mutagens have not been identified. Finally, the biological significance of low-level mutagenicity in urinary concentrates has not been established. Due to these factors there is little reason, at present, to believe that urinary mutagenicity can be used to assess exposure to ETS or to assess risk to cancer [93].

Extrapolation models

An important consideration in the dose-response analysis of risk assessment is the extrapolation model used at the low-dose end of the curve. Traditionally, the linear non-threshold dose-response model has been used in the quantitative risk assessment of carcinogens. Current evidence brings this concept into question [96] and necessitates a rethinking of this process. The theory presented is that, at low doses, initiation may occur but unless exposure to high doses of promoters then occurs, tumours will not develop. This line of reasoning has considerable impact on the procedures used for analysing low-dose exposure as it relates to extrapolation of cancer risk from active smoking to exposure to ETS.

In spite of the distinct differences in dose received from active smoking and exposure to ETS, the extent of exposure to ETS and active smoking has been compared through the use of cigarette equivalents [21,40,43,46,47,65,97]. This approach attempts to convert exposure to ETS into an equivalent exposure from active smoking with the assumption that the risk from ETS exposure is proportionally comparable to the risk from active smoking. This procedure is an oversimplification of the exposure conditions and will provide potentially misleading information [3].

Pathophysiological consequences and implications

As indicated above, it is extremely difficult to extrapolate from active smoking to ETS exposure with any degree of reliability. Similarly, the data do not point to consistent evidence of pathophysiological consequences of ETS based on exposure and dose. Some examples will be presented to illustrate this point.

Several studies have reported that, functionally, smokers may have reduced ventilatory function at rest and a reduced exercise capacity with a greater oxygen debt accumulation [98–101]. For ETS-exposed non-smokers, the effects on ventilatory function and exercise capacity reductions are not consistent. While a few studies show some functional impairment, the majority do not. First of all, it is difficult to determine if the test situation mimics real-life exposure. The conditions to which subjects are exposed are often not relevant to ETS exposure. One study where subjects were passively exposed to cigarette smoke illustrates this point [102]. After drawing the puff through the apparatus consisting of a solenoid, capacity vessel and pump, the MS was discharged into the test room along with the SS. Therefore, the subjects were essentially breathing diluted quantities of the same constituents as an active smoker. The exposure conditions were also rather

extreme. Initial concentrations of particulate matter were $>4 mg/m^3$ and carbon monoxide levels were 24 ppm. After 2 h, the particulate concentration dropped to only $2 mg/m^3$. Therefore, these conditions are not representative of ambient ETS exposure.

Even in this study [102], no change was found in the FEV_1 of the subjects at rest. When bicycle exercise was performed, the only change found was a slight increase in heart rate at two to five time points that was statistically significant but not biologically important.

nother problem in trying to identify possible effects of ETS on pulmonary function is the inaccurate or broad ranges of exposure as represented in either the ETS-exposed or -unexposed groups. Usual confirmation of ETS exposure or lack of active smoking is through questionnaires without chemical confirmation. No matter how limited chemical confirmation techniques are, questionnaires are less reliable. Most epidemiological studies involve spousal exposure and ignore whether smoking occurs in the home to any significant degree or whether spousal exposure is compounded by workplace or social exposure. Intuitively, it might be expected that smokers socialise with others who also smoke more often than do non-smokers. The other major consideration may be tied to the general alth status or awareness of smoker ouseholds compared to non-smoker households. It would seem very important to match groups for diet and exercise as well as other health indicators.

Functional studies

In contrast to the studies reported on MS, it would appear that there is little agreement among studies as to the effects of ETS on pulmonary parameters. Even within studies, unexplainable peculiarities appear that raise questions of reliability. Certain age groups of particular populations are found to be affected where other population segments in the same study show increased pulmonary function capability. In a comprehensive review of this subject the results of studies were regarded as being too variable to permit a conclusion concerning long-term ETS exposure and possible impaired respiratory health or pulmonary function in non-smoking adults [103].

Studies typically are further complicated by the possibility of suggestibility. Suggestibility is the reverse of the placebo effect. These studies are performed to determine the magnitude of the psychosomatic effect and hope to answer the question: "If the subject expects an adverse effect to occur, will this be reflected in a measurable response?" Here again, there is no good agreement. One study reports a 50% increase in airways resistance following a positive suggestion that the subject would be breathing a substance that may be irritating and make it harder to breath [104]. In another study, subjects who could easily tell whether or not they were breathing the smoke, were exercised at a level to increase minute ventilation to about 2.5 times resting ventilation. These subjects showed a dose-related response to sham or zero smoke, and two levels of ETS exposure [105]. The magnitude of change in pulmonary function parameters was minor in most cases and of no physiological significance. The experiment was flawed by the failure clearly to separate the pyschological influence from the physiological effects and to establish any real controls, whereas the previously cited study [104] unquestionably separated the two components. Furthermore, in this study [105] it appeared that all smoke, including the MS generated by the smoking machines, was presented to the subjects.

The question of allergic response to tobacco smoke has been raised frequently, and was investigated by McDougall and Gleich [106], who reported that tobacco and tobacco smoke allergies were not demonstrable. It might thus be concluded that most of the apparent irritation in the presence of ETS is psychologically based.

When considering asthmatic patients, where active smoking has sometimes

been reported to be capable of triggering attacks, the evidence is not well established for ETS. Pulmonary function tests of asthmatics produced no change in expiratory flow rates. However methacholine challenge did produce a slight but significant increase in airway reactivity [107]. Other investigators studied the effects of ETS on asthmatics and found variable and inconclusive results in pulmonary function, but again found the increased reactivity to challenge; this time to histamine [108]. The results seem reasonable; however the regimen was not clearly stated. The mixing of MS and ETS may be a confounding problem of this study, as well. In summary, these results suggest a highly variable functional response to ETS even under laboratory conditions.

Cancer types, locations and frequencies

Use of tobacco products has been reported to be associated with cancers of various types and in various organ systems depending upon the tobacco product used. A review which addresses the comparisons between active smoking and exposure to ETS, concludes that more research needs to be done to demonstrate a strong association between ETS and cancer in the non-smoking population [109].

These authors begin with the hypothesis that the association between ETS and lung cancer must be possible based on the evidence from active smoking. They then examine the criteria set forth in the Surgeon General's report of 1964, and cite the inconsistencies in the results of both prospective and case-control studies. They make a specific point of the necessity for carefully documenting tumours using good histopathological techniques. In their own previously reported and unreported studies, they found that there is a preponderance of Kreyberg type I class tumours associated with smoking. In never-smokers, the preponderance of tumours are classified as Kreyberg type II. Within these catogories the squamous cell type (type I) was predominant in smokers, "with lesser but significant causative effect on the glandular type". In non-smokers, the predominant type is the glandular adenocarcinoma type II tumour. Other authors [110–111] suggest that ETS is limited to squamous cell types of tumours. If this is the case, the numbers of tumours potentially attributable to ETS would be very small considering the low incidence of this type of lung cancer in non-smokers. There is some support for squamous cell tumours being the most likely to be caused by ETS [112], quoted by the US Surgeon General [113]. In a closely monitored study in Olmsted County, Minnesota, Beard and his colleagues found that the incidence rate for squamous cell tumours dropped remarkably in the 1965–1974 period, presumably as smoking decreased. Small cell tumour incidence, also associated with smoking, decreased but not as dramatically. The incidence of adenocarcinoma continued to rise. There are several conclusions that can be drawn:

1. If Dalager *et al.*, [110] and Pershagen *et al.*, [111] are correct in concluding that squamous cell and small cell tumours are the predominant types associated with both smoking and exposure to ETS, then the risk of lung cancer from ETS is very small since this tumour is rare in non-smokers.
2. Since adenocarcinoma of the lung continued to rise in the Olmsted County study and is purported by some investigators to be the predominant type for ETS exposure, the association between ETS and adenocarcinoma is incorrect, meaning that some other cause is associated with the development of adenocarcinoma of the lung.
3. ETS may not, in fact, cause cancer of the lung at all, or if it does, perhaps it is associated with several types of tumours but not at a very high level.

Regardless of who is correct, more careful documentation is necessary of the histological types and incidence of lung tumours in order to determine an accurate and meaningful risk.

Conclusions

Since ETS has not been adequately characterised, there are insufficient data on which to base a hazard analysis. Accordingly, there are not enough data available on which to base an exposure assessment for ETS. Due to the dynamic nature of ETS, it is impossible to relate ETS to MS chemically or physically. In the absence of this relationship, it is inappropriate to make any extrapolations from what is reported about the effects of active smoking to possible effects of exposure to ETS. Therefore, any calculation of risk from exposure to ETS based on extrapolations from calculated risks of active smoking is, at best, not reliable and, most probably, of no value whatsoever. It is important, therefore, to consider ETS as a distinct entity, and further research is needed to test hypotheses based on valid protocols that meet the criteria established for the epidemiology of weak associations.

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